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Respuesta ecofisiológica del espartal mediterráneo semiárido al cambio climático

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PhD Thesis

Mónica Ladrón de Guevara Sáez de Eguílaz

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TESIS DOCTORAL

Ecophysiological response of semiarid Mediterranean grassland to climate change

Respuesta ecofisiológica del espartal mediterráneo semiárido al cambio climático

Memoria presentada por Mónica Ladrón de Guevara Sáez de Eguílaz para optar al Grado de Doctor en Ciencias Aplicadas al Medio Ambiente por la Universidad de Almería.

Esta tesis ha sido dirigida por Roberto Lázaro Suau, Científico Titular de OPIs y Francisco Domingo Poveda, Investigador Científico de la Estación Experimental de Zonas Áridas, (CSIC).

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PRÓLOGO

A modo de ejemplo, algunos especímenes encontrados en esta tesis:

INFELICIDÁCEA (Infelicitas infelix), invisible planta perenne cuyo tallo varía desde un par de milímetros hasta varios metros; es de hojas grandes, carnosas, con abundante vello y bordes dentados de manera irregular. La Infelicidácea se reproduce con gran rapidez sin importar la estación del año. Tiene el poder de regeneración, así que del más minúsculo pedacito de esta planta puede desarrollarse una nueva. Contiene un jugo venenoso cuyo efecto es lento y doloroso y causa ampollas que estallan y crean heridas difíciles de sanar.

FELICIDÁCEA (Felicitas felix), invisible planta anual (pocas veces perenne) de desigual altura; es de hojas redondas, delgadas y suaves como la seda. La Felicidácea crece en todos los continentes, pero a diferencia de la Infelicidácea requiere de condiciones climatológicas moderadas y un cuidado entregado. El jugo de la Felicidácea es curativo, conocido desde los tiempos más remotos como un medio exitoso contra muchas enfermedades. La flor abierta de esta planta es de una hermosura tan espléndida que embellece todo a su alrededor inmediato. Tiene un aroma sumamente agradable.

<<La Infelicidácea y la Felicidácea >>, del herbario Flora invisible, folio 10284/ folio36477. Departamento de Botánica de la Academia para las Invisibilidades, Leningrado.

Extracto literario de *Atlas descrito por el cielo,* Goran Petrović

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ÍNDICE

CHAPTER III

General introduction

GENERAL INTRODUCTION

1. Statement of the problem

Global change is a broad concept without easily defined boundaries that can encompass several planetary-scale changes in the Earth system, such as atmospheric and ocean circulation; climate; the carbon, nitrogen and water cycles; sea-ice and sea-level changes; food webs and stocks; biological diversity etc. Since the Industrial Revolution, humans have been a large driver of global change, and their great influence on the Earth system is increasingly recognized. The term is gradually taking on a social dimension, including issues such as pollution, health, population, the economy, resource use, energy, development, transport, communication, land use and land cover, urbanization and globalization (IGBP, online web). Therefore, climate change is only one component of the complex global processes that operate in the Earth system but with a great capacity to interact with the rest of the elements of the system. The Mediterranean Europe regions have been identified as one of the most prominent climate response hot spots, i.e., there are regions where potential climate change impacts on the environment or different activity sectors can be particularly pronounced (Giorgi, 2006). The Mediterranean is a transition region between the dry-hot North African climate and the wetter-cooler European climate, and for this reason, it has a particular sensitivity to minor modifications to the general circulation of air masses (Giorgi & Lionello, 2008; Mariotti et al., 2015). Precipitation patterns are the most evident limiting factor for human activities and natural ecosystems in the Mediterranean (Luterbacher et al., 2006), and their concurring feature is a high degree of variability. Therefore, it is necessary to determine how the Mediterranean ecosystems will respond to predicted climate change by the middle of this century, especially in rainfall patterns, in order to cushion its impact and support decision making in land management with science-based knowledge.

1.1. Forecasted climate change in the Western Mediterranean Basin and its impacts on terrestrial ecosystems

Long-term twenty-first-century projections show a warmer and drier climate in the Mediterranean Basin (De Castro et al., 2005; Cook et al., 2014; Mariotti et al., 2015), in

line with the tendencies observed in the past century (Hoerling et al., 2012; Kelley et al., 2012). The confidence in temperature model projections is high, with a very likely increase exceeding 4–5ºC over the course of this century and with more frequent extreme temperature events (Giorgi & Lionello, 2008; IPCC, 2012; Christensen et al., 2013). Changes in the water cycle remain less reliably modeled, but there is a consensus in predicting changes in precipitation patterns and an intensification process of rain events (Stocker et al., 2013). Precipitation in the Mediterranean Basin is projected to decrease in amount and frequency throughout the year but with greater intensity during the dry season, exceeding a reduction of about 25–30% (Giorgi & Lionello, 2008; Christensen et al., 2007, 2013). However, the decrease in effective land precipitation (precipitation – evaporation) will be similar during the wet and dry seasons (Mariotti et al., 2008). In addition, an intensification of extreme precipitation events for all seasons has been projected at the end of the present century as well as an increase of the frequency and duration of drought situations, with general soil moisture drying in the Mediterranean (IPCC, 2012; Toreti et al., 2013). In conclusion, all these changes will cause an intensification of the water cycle, i.e., an acceleration of the main processes of this cycle, such as precipitation and evaporation (Huntington, 2006; Stocker et al., 2013), leading to a transition from Mediterranean to arid climate in various regions of the Mediterranean coast (Alessandri et al., 2014).

Indeed, these forecasts have already been observed. The last IPCC reports point to a very likely increase in the number of warm days and nights and a decrease in the number of cold days and nights since 1950 in Europe (Christensen et al., 2013; Hartmann et al., 2013). There is a clear trend toward drier conditions in most western Mediterranean regions (Sousa et al., 2011; van den Besselaar et al., 2012), with an increase of the frequency and intensity of droughts, extreme precipitation and floods (Zolina et al., 2009; Hoerling et al., 2012; Hartmann et al., 2013; Stocker et al., 2013). Ocean and sea salinity patterns of the last century are also a clear indicator of the intensification of the global water cycle (Huntington, 2006; Durack et al., 2012). As an example, in the Iberian Peninsula, dendrochronological (Puigdefábregas & Mendizabal, 1998), lacustrine (Martinez-Cortizas et al., 1999; Luque & Julià, 2002) and coastal marine records (Diz et al., 2002; Desprat et al., 2003) have allowed characterizing the climate between the fourteenth and nineteenth centuries, denominated as the Little Ice Age (de Castro et al., 2005), as a cooler and wetter period compared with the current climate. The last cold pulse of this period took place in the early nineteenth century

(Chueca et al., 2003), and since then, tree rings of Sierra de Cazorla, in the southern peninsula, and of the Pyrenees, in the north, have showed a slow increase of temperatures and a decrease of fall and winter rains (Puigdefábregas & Mendizabal, 1998). The observed changes in the climate system are unlikely to be entirely caused by natural variability (Rosenzweig et al., 2008). In addition to the natural forcing (via solar irradiance changes and volcanic aerosols) in the industrial era, anthropogenic forcing has become the main climate change driver through the atmospheric increase of well-mixed greenhouse gases and changes in land uses (Myhre et al., 2013).

Climate changes have consequences, only partially known, on terrestrial ecosystems, and they will be intensified in the near future (Sala et al., 1999). These effects include shifts at different scales: ecosystemic (e.g., biogeochemical cycles, community structure, trophic webs), population (e.g., phenology, distribution, extinction rates) and even at the genomic scale (Mouillot et al., 2002; Parmesan, 2006; Jump et al., 2008; Gordo & Sanz, 2010; Jochner & Menzel, 2015). Despite an abundance of evidence demonstrating the adaptation of many populations to their current environment, there is a knowledge gap about how local adaptation of populations will interact with a fast climate change (Jump & Peñuelas, 2005) and what consequences this will have in the ecosystem functions and climate-vegetation feedbacks.

2. State of the art and thesis framework

2.1. Climate-vegetation relationship

The relationship between climate and the distribution of different vegetation types is an important unifying principle in ecology (Stephenson, 1990), as several climatic variables, such as seasonal rainfall and water balance; the length of growing seasons; or annual, maximum and minimum temperatures and their timing, can strongly influence the physiology of plants and their survival (Prentice et al., 1992; Foley et al., 2003; Seppä et al., 2015). It is possible to find illustrative evidence of this relationship, such as, for example, evolutionary convergence of plant phenotypes phylogenetically unrelated but with a similar climatic history (Mooney & Dunn, 1970; Mooney, 1977; Cody & Mooney, 1978) or alterations in species and biomes as a result of past climate changes (Delcourt & Delcourt, 1983; Davis, 1983; Prentice, 1986; Tarasov et al., 1998; Claussen et al., 1999; Clark et al., 2001; Kullman, 2013; Seppä et al., 2015). Thus,

climate variables determine the characteristics of the land surface; and this last, in turn, affects the fluxes of energy, CO_2, H_2O and particles that modulate meteorological processes and the physical climate system (Sellers & Schimel, 1993; Nicholson, 2002; Foley et al., 2003).

Consequently, most climate classification systems incorporate vegetation-climate relationships (Emanuel et al., 1985), and it is not surprising that in recent decades and currently, one of the most recurrent research topics is the alteration of the structure, distribution and functionality of vegetation by climate change (Loehle & LeBlanc, 1996; Cramer et al., 2001; Grimm et al., 2013) and vegetation-climate feedbacks (Schlesinger et al., 1990; Field et al., 1992; Foley et al., 2000, 2003; Bonan et al., 2003; Melillo et al., 2001; Sitch et al., 2003; Sciortino, 2011; Aguirre-Salado et al., 2012). Climate change will very likely affect fluxes resulting from biophysical and biogeochemical processes at the land-atmosphere boundary and, as a result, will create positive or negative feedback effects. Examples of these processes, pointed out by Ojima et al. (1991), include (i) net C fluxes via soil and plant heterotrophic and autotrophic activity, (ii) biogenic trace gas exchange controlled by nutrient cycling and (iii) water and energy fluxes via evapotranspiration, runoff and run-on. Feedback effects also involve ecosystem cumulative changes that impact on natural resources upon which humans depend, so climate change, in addition to being a topic of current scientific interest, has a social dimension whose consequences are difficult to assess.

2.2. Climate manipulation experiments as a method in this thesis: a brief review of their use

Climate manipulation experiments are one of the most useful tools to explore the ecological impacts of climate change, and they have contributed significantly to our understanding of ecological principles over the past few decades (Fraser et al., 2013). Since the 1980s, climate manipulation experiments have experienced a boom, especially regarding the response of vegetation to increased temperature and/or concentration of CO_2 and nutrients in different environmental gradients as well as human disturbance. These researches have been carried out mainly in arable crops (Rogers et al., 1983; Bhattacharya et al., 1988, 1990; Hendrey et al., 1993; Lewin et al., 1994; Adam et al., 2000; Kim et al., 2001; Okada et al., 2001; Leakey et al., 2004) and forest plantations (Rogers et al., 1983; Ellsworth et al., 1995; DeLucia et al., 1999; Oren et al., 2001;

Wullschleger & Norby, 2001; Norby et al., 2004) for their socioeconomic implications; and in boreal (Chapin III & Shaver, 1985, 1996; Tissue & Oechel, 1987; Chapin III et al., 1995; Michelsen et al., 1996; Hobbie & Chapin III, 1998; Molau & Alatalo, 1998; Shaver et al., 1998; Wright & Jenkins, 2001), alpine and subalpine ecosystems (Harte & Shaw, 1995; Loik & Harte, 1996; Klanderud & Totland, 2005; Klanderud, 2008; Jägerbrand et al., 2009) since they have been identified by the IPCC as natural systems at risk of irreversible damage (McCarthy et al., 2001). Attention has also been given to temperate forests (Karnofsky et al., 2003; Keel et al., 2006), temperate and semiarid grasslands (Jackson et al., 1994; Hu et al., 2001; Morgan et al., 2004; Bachman et al., 2010), temperate steppes (Niu & Wan, 2008; Niu et al., 2008; Xia et al., 2009) and aquatic ecosystems (Berendse et al., 2001; Heijmans et al., 2001; Miglietta et al., 2001).

In these experiments, warming is accomplished using different methods: heating cables attached to the soil, open- or closed-top chambers, greenhouses, overhead infrared heat lamps and passive nighttime warming using reflective curtain covers (Rustad et al., 2001; Wu et al., 2011). The most common methodology to achieve an atmospheric increase of CO_2 concentration is the Free Air CO_2 Enrichment (FACE) system, which consists of a blower connected to a horizontal and circular pipe with several vertical vent pipes. Pure CO_2 is injected in the blower-circular pipe connection, and a turbulent mixing of the ambient air and the injected CO_2 exits to the experimental plots through the vertical pipes (Miglietta et al., 2001).

However, shifts in rainfall patterns are a climate change component less explored until recent years, in spite of the alteration of water availability is virtually certain to affect terrestrial ecosystem processes, particularly in those ecosystems where this resource is already limited, as water is critical for life (Miranda et al., 2011; Wu et al., 2011; Vicca et al., 2012). Studies about rainfall shifts in semiarid ecosystems were also anecdotal until the last decade, although these ecosystems cover about 30% of the land area (Le Houérou, 1996; Viles, 2008), and precipitation and water availability have stronger influence over the processes of these systems (for example, soil respiration, gross and net ecosystem productivity, nutrient cycling, plant recruitment, growth and reproduction) than the isolated or combined effects of changes in CO_2 concentration and temperature (Weltzin et al., 2003; Shen et al., 2009). This lag of knowledge has been pointed out in this century (Weltzin et al., 2003; Miranda et al., 2011; Gherardi & Sala, 2013; Zeppel et al., 2013; Nielsen & Ball, 2015), and in recent years, rainfall manipulative studies (as a single factor or in combination with others) are increasing in

dryland regions (see definition in next section), focusing on vegetation, soil biota and their processes (e.g., Bates et al., 2006; Darby et al., 2006; Potts et al., 2006a, b; Patrick et al., 2007, 2009; Cipriotti et al., 2008; Heisler-White et al., 2008, 2009; Shen et al., 2008; Miranda et al., 2009a, b, 2011; Niu et al., 2009; Evans et al., 2011; Jongen et al., 2011; 2013a, b, 2014; Thomey et al., 2011, 2014; Yang et al., 2011; Coe et al., 2012; Escolar et al., 2012, 2015; Johnson et al., 2012; Martínez-García et al., 2012; Reed et al., 2012; Yeager et al., 2012; Zelikova et al., 2012; Báez et al., 2013; Byrne et al., 2013; Rico et al., 2013; Barbeta et al., 2015; Padilla et al., 2015).

The goals of rainfall manipulative experiments consist in altering water amounts with decreased and/or increased rainfall treatments. Decreased rainfall treatments can completely remove selected events (from a particular season or all annual events) by means of rainout shelters with fixed or movable roofs or water-excluding curtains that cover the totality of the experimental plots (e.g., Beier et al., 2004; English et al., 2005; Harper et al., 2005; Grime et al., 2008; Mikkelsen et al., 2008; Albert et al., 2011; Evans et al., 2011; Jentsch et al., 2011; Selsted et al., 2012; Báez et al., 2013) or only remove a fraction of all events of the experimental period by means of fixed rainout shelters that partially cover the plots (e.g., Yahdjian & Sala, 2002; Smith, 2011; Escolar et al., 2012, 2015). The aim of the manipulation can also be altering the frequency and/or seasonal distribution of rainfall with (e.g., Fay et al., 2000; Miranda et al., 2011; Padilla et al., 2015) or without variation of the total monthly or annual amount (e.g., Laporte et al., 2002; Belnap et al., 2004; Bates et al., 2006; Heisler-White et al., 2008; Volder et al., 2013; Jongen et al., 2013a, b, 2014). Sometimes, irrigation implementations are necessary when the simulation of different rainfall patterns requires the total removal of natural rainfall during an annual or seasonal period of time (e.g., Fay et al., 2008; Heisler-White et al., 2008; Miranda et al., 2009a; Volder et al., 2013). In these cases, the experiment requires the use of irrigation systems, hand sprinklers and rain collectors with gutters (Wu et al., 2011). However, maybe because of more methodological complexity, there are fewer studies that cross as independent factors the amount and frequency of rainfall (Fay et al., 2000, 2002, 2003, 2008, 2011; Harper et al., 2005; Miranda et al., 2009a, b), and only Miranda et al.'s studies were implemented in semiarid ecosystems. With an automatic movable shelter, this thesis addresses the difficulties of this experimental design, and with the use of fixed passive shelters and open-top chambers, additional goals listed in the following main section (objectives and justifications) have been carried out.

2.3. Target ecosystem and its climate

The experiments implemented in this thesis were located in semiarid Mediterranean grasslands. Definitions for grasslands vary according to the approach. Some studies use primarily the vegetation type, while others characterize them by climate, soils and human use of the ecosystem (White et al., 2000). The approach based solely on vegetation type is the most used by the remote-sensing community, and in these terms, grasslands are defined as lands with herbaceous types of cover with less than 10% of tree and shrub cover (Woodward et al., 2001). In addition to vegetation characteristics, several authors further consider that this ecosystem is maintained by fire, grazing, drought and/or freezing temperatures (Anderson, 1982; White et al., 2000; King, 2010; Unger et al., 2015). Indeed, in the last 30 to 45 million years, grasses have experienced rapid spread and diversification thanks to the combination of two main drivers: an increase of global aridity and human disturbance by means of fire and grazing (Woodward et al., 2004). Using a land cover classification based on the aridity index (ratio of mean annual precipitation to potential evapotranspiration) of UNEP (1992), currently most of the grasslands are located in semiarid zones (28%), followed by humid (23%), cold (20%), arid (19%), dry subhumid (8%) and hyperarid zones (2%), covering in total around 31–43% of the Earth's land surface (White, et al., 2000; Unger & Jongen, 2015).

Hyperarid, arid, semiarid and dry subhumid ecosystems occupy 41% of the Earth's land surface and as a whole are called drylands (Safriel & Adeel, 2005; Mortimore, 2009). These areas support 35% of the human population and around 50% of livestock (Puigdefábregas & Pugnaire, 1999; Mortimore et al., 2009). As pointed out above, the largest spread of grasslands is found in semiarid regions, where the target ecosystem of this thesis is located. Based on the annual rainfall, Noy-Meir (1973) defined semiarid ecosystems as those that have a lower range of precipitation located between 150 and 250 mm and an upper range between 250 and 500 mm. One way to identify the boundary between arid and semiarid areas is by considering the field capacity of land use, as diffuse dryland farming is becoming feasible in semiarid areas. The climax vegetation of these ecosystems consists of sclerophyllous shrubs and small trees of relatively slow growth. Where productivity is even lower, secondary succession is very slow, moving from a poor coverage of annual plants to vegetation coverage

composed of slow-growing herbaceous perennial plants and scattered woody plants (Grime, 2002).

The grassland biome can be divided into the following categories: flooded, montane, temperate, tropical and subtropical. Within this last category is included the Mediterranean grassland biome, where vegetation communities under the influence of this particular climate are present. Mediterranean climate is characterized by mild to cold, wet winters and warm to hot, dry summers (Lionello et al., 2006). Thus, this climate has a strong seasonal distribution of precipitation, where severe droughts prolonged during three or four months coincide with the warmest season (Moreno et al., 2008) and the most important period of rainfall normally coincides with the coldest temperatures. A great portion of Mediterranean ecosystems are located in semiarid regions, but they are not exclusive to these areas since this climate has a humid boundary around 900 mm (Aschmann, 1973). Besides the enhanced seasonal differences, another characteristic of the Mediterranean climate is the strong interannual variability of precipitation (Paredes et al., 2006). The variability of the wet season precipitation amount has a primordial influence on the hydrological budget of the areas under this climatic regime and therefore also has a great impact on their natural, social and economic systems (Xoplaki et al., 2004). In conclusion, grasslands under Mediterranean climate have to be adapted to seasonal drought, but they are vulnerable to decreased precipitation since the soil water store is limited. These grasslands are typically dominated by C_3 annual species and drought-resistant perennials that have a beginning of the growing season marked by the timing of the first precipitation in autumn. However, their maximum activity does not occur until winter or early spring, and the senescence generally starts in May (Jongen et al., 2011).

Regions under Mediterranean climate and grassland ecosystems are identified as the hot-spot areas regarding biodiversity changes due to the convergence of the main biodiversity shift drivers, such as climate change, land-use changes, nitrogen deposition, biotic exchange and elevated CO_2 concentration (Sala et al., 2000), but the main driver will likely be water availability. For example, grasslands in drylands have shown in the past species-specific sensitivity to forecasted climate change effects, such as extreme drought events (Weaver & Albertson, 1943), affecting grassland composition and productivity (Clark et al., 2002), and Schlesinger et al. (1990) suggest that the increase of spatial and temporal heterogeneity of soil resources, especially moisture, will likely lead to the degradation of semiarid grasslands. Perhaps because of the more complex

management and spatial heterogeneity of grasslands, the potential effects of climate change in them have been less studied than in forests until recent years. However, this ecosystem plays an important role in the global carbon cycle since it contains about 10% of global carbon stocks (Solomon et al., 1993; Jones, 1997). Overall, the selected ecosystem type represents areas of special interest in the study of their interaction with the future climate scenarios, in addition to being the most widespread ecosystem in the Western Mediterranean Basin. The progress in the knowledge of this ecosystem functioning represents a fundamental key for understanding the overall dynamics of terrestrial ecosystems and climate feedback effects.

2.4. Target organisms and justification of the selection

2.4.1. Macrochloa tenacissima (L.) Kunth (= Stipa tenacissima L.), (common name alpha grass, esparto)

M. tenacissima is one of the most frequent and characteristic species in the driest areas of the Western Mediterranean ecosystems, and it is usually dominant in its community, forming a steppe ecosystem, so knowing how this species will respond to forecasted environmental changes could give information about the ecosystem response. This species is a C_3 herbaceous perennial plant that covers currently about 5.9 million ha in Northwest Africa and the Iberian Peninsula. It occurs between the 100 and 600 mm isohyets but is especially abundant in areas with 200–400 mm per year (Maestre et al., 2007; Le Houérou, 2009). In the late 19th century, *M. tenacissima* steppes were extended to about 8 million ha, but human activities, such as clearing for cultivation, overexploitation for the paper industry and burning for grazing, have caused their regression and degradation (Le Houérou, 2001, 2009). The importance of this species is reflected not only in terms of spatial dominance in the Mediterranean semiarid environments but also because of its remarkable morphophysiological adaptations to this climate (Ramírez, 2006). Under the apparent homogeneity of these steppes, there is relatively high biodiversity due to the variety of microenvironments generated by *M. tenacissima* (Sánchez, 1995; Ghilouf et al., 2015). This species also has different ecological functions, such as its potential (i) to increase soil retention moisture, soil organic carbon, resilience against erosive forces and interception of sediments from runoff, promotion of the conservation and development of soil and configuration of

islands of fertility (Sánchez, 1995; Puigdefábregas & Sánchez, 1996; Cerdà, 1997; Maestre & Cortina, 2004; Mora & Lázaro, 2013; Berdugo et al., 2014) and (ii) to facilitate the establishment of woody species that ameliorate water infiltration and nutrient cycling (García-Fayos & Gasque, 2002; Maestre et al., 2003; Maestre & Cortina, 2004).

2.4.2. Biological soil crusts

For decades, ecologists have highlighted the important role of biological soil crusts (hereafter BSCs or biocrusts) in dryland management (e.g., Cundell, 1977; Anderson et al., 1982; Brotherson et al., 1983; Fleishner, 1994; Belnap & Gillette, 1998; Harper & Belnap, 2001; Belnap & Lange, 2003; Bowker, 2007). BSCs can cover up to 70% of the surface in some ecosystems of arid and semiarid regions (Belnap, 1994; Belnap & Lange, 2003). They are made up of an intimate association between soil particles and cyanobacteria, green algae, heterotrophic bacteria, microfungi, lichens and bryophytes distributed in the topsoil (Belnap et al., 2003a; Pointing & Belnap, 2012), constituting an important component in the target ecosystem of this thesis, as these organisms frequently occupy plant interspaces. BSCs have many roles in the formation, stabilization and fertilization of soils, for example, promoting the stability of dunes and the surface and subsurface soil layers through the formation of a network of fine filaments that interact with sand and soil particles (Fearnehough et al., 1998; Li et al., 2002; Belnap, 2003), creating stronger sand and soil aggregates through the production of extracellular polysaccharides (Belnap & Gardner, 1993; Belnap et al., 2005; Chamizo et al., 2012a; Wu et al., 2014); protecting soil surface from wind and water erosion (Belnap, 1994; Verrecchia et al., 1995; Belnap & Gillette, 1998; Rodríguez-Caballero et al., 2012; Lázaro & Mora, 2014); favoring the deposition of nutrient-rich dust, organic matter and soil fines due to their complex microtopography (Reynolds et al., 2001; Belnap et al., 2003b); or acting as nitrogen fixers (West, 1990; Evans & Ehleringer, 1993; Belnap, 2002; Zhao et al., 2010).

BSCs can also influence several soil properties that determine local hydrologic cycles (Belnap, 2006; Chamizo et al., 2012b, c, 2013; Lázaro et al., 2014) and are linked with the carbon cycle as primary production contributors. In spite of the fact that in drylands carbon stocks in soil are at least twenty times higher than in vegetation (Grainger et al., 2000; Eglin et al., 2010), the specific importance of BSCs as carbon

fixers has rarely been estimated in the field until recent years (Brostoff et al., 2005; Elbert et al., 2012; Raggio et al., 2014), and climate manipulative experiments over them are also scarcer than in other functional types, maybe because, due to their conditions of poikilohydric and poikilothermic organisms, they are supposed to be unaffected (or hardly affected) by climate change. The effects of BSCs on vascular plants are probably very diverse, including negative and positive interactions. The observable positive effects include an increase of soil water and bioessential element availability in the soil (Mayland et al., 1966; Harper & Pendleton, 1993; George et al., 2003), improving in this way the nutritional status of the plants (Harper & Belnap, 2001), secretion of substances that promote growth and cell division in plants, or an increment of plant survival and biomass (Belnap et al., 2003b). On the other hand, thickened crusts can prevent the germination and colonization of many plant species by not permitting direct soil contact with the seeds and thereby limiting their extension, density and diversity (McIlvanie, 1942; Prasse & Bornkamm, 2000; Li et al., 2002; Funk et al., 2014). This negative interaction is functional type specific, with stronger impact on shrubs and perennial plants than on grasses and annuals (Mitchell et al., 1998; Li et al., 2002; Langhans et al., 2009). In view of the above considerations, the functioning of drylands is greatly influenced by BSCs, and their study is receiving increasing attention.

3. Objectives and justifications

The main objective of this thesis is to estimate the effects of climate change on the ecophysiology of two key plant functional types of semiarid ecosystems: the dominant grass *Macrochloa tenacissima* and BSCs. As it has been explained above, Mediterranean semiarid regions may be among the most affected by climate change, being drylands very vulnerable to degradation processes (Sciortino, 2011). They are a transitional biome between temperate forests and deserts (Lavorel et al., 1998), with large annual rainfall fluctuations and with many species close to their bioclimatic limits, so small changes in climate could lead to large latitudinal or altitudinal displacements of species distribution limits. Furthermore, given the great extension of drylands and the important human and livestock population that they support, knowledge of the expected changes in them has a considerable socioeconomic interest, in addition to being critical to understanding the overall dynamics of terrestrial ecosystems and their feedback with

climate. Therefore, these regions under a high pressure of human activity need proper land resources management policies considering the future ecosystem shifts driven by global warming.

Water is a primary resource limiting terrestrial biological activity (Huxman et al., 2004a), especially in arid and semiarid ecosystems, where erratic moisture inputs impose a pulsed pattern on biological processes (Noy-Meir, 1973; Weltzin et al., 2003; Collins et al., 2008). Hence, rainfall is the main climatic factor that controls several ecosystem functions and dynamics in these regions, such as land productivity and decomposition activity (Carrera et al., 2008; Karrou et al., 2011; Gicheha et al., 2012; Uchehara, 2013). For this reason, in this thesis, the climate manipulative experiment using *M. tenacissima* has been focused on predicted shifts in precipitation patterns as the main driver of the alterations of ecophysiological functions due to climate change (**Chapters I and II**). Several authors showed that patterns of monthly and seasonal precipitation are at least as important as the amount of annual precipitation (Xiao et al., 1995; Bates et al., 2006; Robertson et al., 2009), as the timing and frequency of precipitation can have large effects (Knapp et al., 2008). However, as noted above, there are few rain exclusion studies that include the effects on vegetation and soil activity of changes in both rain amount and frequency. In addition, several studies pointed out that extreme events play an important role in growth performance, physiology and population dynamics of vegetation and soil biota (Gutschick & BassiriRad, 2003; De Boeck et al., 2011; Jentsch et al., 2011; Kreyling et al., 2011; Lloret et al., 2012). Overall, the determination of *in situ* ecophysiological responses (net photosynthesis, stomatal conductance, intrinsic water use efficiency and F_v/F_m) of *M. tenacissima* to rainfall changes has been focused on two shift factors, the reduction of quantity and frequency of rainfall (**Chapter I**). Rain shifts caused by the latter factor included an intensification of the extreme events.

Besides, Luo et al. (2011) defined three main components that interactively determine long-term ecosystem responses to global change: (i) climate variability, (ii) disturbance and (iii) internal long-term processes. Hence, the knowledge of the natural variation of a system as a response to the current climate variability is a necessary step in understanding ecological processes and the implications of ecological changes (Landres et al., 1999). As is highlighted above, semiarid Mediterranean climate is characterized by great intra- and interannual variability in its precipitation, and *M. tenacissima* has developed several morphological and physiological strategies to cope

with this environment of irregular water inputs (Pugnaire & Haase, 1996; Pugnaire et al., 1996; Haase et al., 1999; Valladares & Pugnaire, 1999; Balaguer et al., 2002; Domingo et al., 2002; Ramírez et al., 2006, 2007, 2008a, b; Armas et al., 2009; Boscaiu et al., 2009; Chirino et al., 2011). Another goal of this thesis is to determine whether the increment of rainfall variability forecasted for the Mediterranean Basin is a force of change powerful enough to drive *M. tenacissima*'s physiological responses beyond its current variation range (**Chapter I**).

Most of the literature indicates a positive correlation between productivity and annual or growing season total amount of rainfall (Le Houerou & Hoste, 1977; Knapp & Smith, 2001; Huxman et al., 2004a; Nippert et al., 2006; Hsu et al., 2012). However, in this century, the idea that discrete rainfall event features play a main role in the C cycle of drylands is gaining prominence (Austin et al., 2004; Huxman et al., 2004b; Schwinning & Sala, 2004; Schwinning et al., 2004; Shen et al., 2008; Chen et al., 2009; Hao et al., 2013). Therefore, using *M. tenacissima* as the target species, the main objective has been addressed in different temporal scales: annual, seasonal (**Chapter I**) and single-event scale (**Chapter II**). Besides, since ecosystems have the capacity to store water, carbon, and nitrogen in various pools and the sizes of some of these pools also influence plant responses to new precipitation inputs, ecosystems and plants clearly have a "memory" of past precipitation events (Ogle et al., 2004; Schwinning et al., 2004). Therefore, **Chapter II** seeks to analyze the response thresholds of *M. tenacissima*'s net photosynthesis to discrete rain events, taking into account the characteristics of the previous rainfall.

Infrared gas analyzers have been used for decades to study CO_2 exchange in several types of organisms, such as vascular plants, cyanobacteria, lichens, mosses and algae (Harris, 1971; Bloom et al., 1980; Dring & Brown, 1982; Schipperges & Rydin, 1998; Brostoff et al., 2002; Millan-Almaraz et al., 2009). However, commercial chambers coupled to these systems are mainly focused on vascular plants. Therefore, to pursue the main objective in BSCs as target organisms, previously, the need has arisen to design, build and test a custom chamber that better matches the experimental requirements: (i) to measure *in situ* CO_2 on soils covered by BSCs with minimal physical and microenvironmental disturbance, (ii) to acquire CO_2 exchange measurements comparable with the most widely employed systems and methodologies and (iii) to be able to monitor the same samples over time (**Chapter III**).

As noted above, BSCs can cover up to 70% of the surface in some ecosystems of arid and semiarid regions. In the geographic framework of this thesis, they are often the second biotype in terms of coverage, occupying the space between *M. tenacissima* individuals. Global net carbon uptake by cryptogamic ground cover has been estimated at about 2.5 Pg yr^{-1} (Elbert et al., 2012), which corresponds to around 4.5% of net primary production by terrestrial vegetation. Their maximum net photosynthetic rates under optimum conditions are highly variable, from 0.1 to 11.5 µmol CO_2 m^{-2} s^{-1} (Burgheimer et al., 2006), and are greatly influenced by the species composition and their proportion (Castillo-Monroy & Maestre, 2011). Traditionally, most of the information about photosynthesis and respiration of lichens and BSCs was obtained in *ex situ* conditions (e.g., Lange, 1980; Lange & Tenhunen, 1981; Kappen, 1983; Kappen & Friedmann, 1983; Lange & Redon, 1983; Ino, 1985; Lange et al., 1986, 1988, 1992, 1995, 1997, 1998; Sancho & Kappen, 1989; Nash III et al., 1990; Cowan et al., 1992; Friedmann et al., 1993; Scheidegger et al., 1995; Schroeter & Scheidegger, 1995; Cable & Huxman, 2004; Grote et al., 2010). Although it has been seen that these types of studies show valuable information to interpret the responses of lichens and BSCs in their natural habitats (Lange et al., 2001), they also present uncertainties in determining if such *ex situ* responses have significant biases to those produced in their places of origin. For this reason, currently, an increasing amount of researches are performing these measurements *in situ* (Yan-Ghi et al., 2013; Li et al., 2012; Su et al., 2012; Wu et al., 2015). In addition, recent studies showed that soils covered by BSCs have higher CO_2 emissions than bare soils, although the rates are dependent on the dominant organism (Castillo-Monroy et al., 2011; Zhao et al., 2014). This fact increases the interest to determine in which environmental conditions BSCs act as a source or as a sink of CO_2. Overall, the aim of this thesis is the determination of *in situ* ecophysiological responses (net and gross photosynthesis, respiration and F_v/F_m) of soils with two levels of BSC coverage to increased temperatures and decreased rainfall (**Chapter IV**).

We have considered it appropriate to include in these target organisms a temperature treatment with a full factorial combination with rainfall and coverage treatment because (i) it is technically feasible to manipulate the temperature of a sufficient representation of the biological community due to the small size of the organisms; (ii) there is evidence that temperature affects at least the activity of soil heterotrophic microorganisms, although this relationship is mediated by soil moisture

(Davidson et al., 1998; Reichstein et al., 2003; Rey et al., 2011; Shen et al., 2015); (iii) due to the poikilohydric nature of the largest proportion of organisms that make up the crust and the very small amount of water that they need to activate their metabolism, the partial exclusion of rainfall could have little effect on them and iv) the experimental design used in this thesis was already being implemented by researchers from the Rey Juan Carlos University, so a common design will enable us to obtain added value of the measurements performed in a climatic gradient with the same treatments. For a better understanding of the temporal linkages between abiotic events and biotic responses of biologically crusted soils, the surveys were performed in two temporal levels: daily and seasonal (**Chapter IV**).

Summarizing: the main purpose of this thesis is to estimate the effects of climate change on the ecophysiology of two key plant functional types of semiarid ecosystems: the dominant grass *Macrochloa tenacissima* and BSCs. Concrete partial objectives were:

1. To determine *in situ* ecophysiological responses (net photosynthesis, stomatal conductance, intrinsic water use efficiency and F_v/F_m) of *M. tenacissima* to experimental rainfall changes focused on two main reduction factors (quantity and frequency of rainfall) and their interaction (**Chapter I)**.
2. To determine whether the increment of rainfall variability forecasted for the Mediterranean Basin is a force of change powerful enough to drive *M. tenacissima*'s physiological responses beyond its current variation range (**Chapter I**).
3. To explore the consistency of the physiological responses on three time scales: annual, seasonal (**Chapter I**) and single-event scale (**Chapter II**).
4. To describe the response thresholds of *M. tenacissima*'s net photosynthetic activity to rainfall attributes that consider the characteristics of the last single event and the antecedent rainfall conditions (**Chapter II**).
5. To design, build and test a custom chamber to measure *in situ* CO_2 exchange on soils covered by BSCs in a standard way to be able to monitor the same samples over time (**Chapter III**).

6. To determinate *in situ* ecophysiological responses (net and gross photosynthesis, respiration and F_v/F_m) of soils with two levels of BSC coverage to increased temperatures and decreased rainfall (**Chapter IV**).
7. To distinguish between daily and seasonal biotic responses of biologically crusted soils to the experimental climatic changes to better understand the temporal linkages (**Chapter IV**).

8. To verify whether climate change effects on the tested variables vary with the degree of BSC development (**Chapter IV**).

After these four chapters, a general discussion and conclusions are presented with the aim of comparing the responses found in the two functional types selected as representative of the whole vegetation and providing a more global perspective of the possible dynamic of the target ecosystem under the simulated climate change scenarios. This knowledge offers an opportunity to evaluate the level of vulnerability of semiarid Mediterranean grasslands to climate change as a first step to select the best management tools to mitigate the most likely impacts.

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Chapter I

Climate change effects in a semiarid grassland: physiological responses to shifts in rain patterns

Ladrón de Guevara, M., Lázaro, R., Arnau-Rosalén, E., Domingo, F., Molina-Sanchis, I. & Mora, J.L. (2015). Climate change effects in a semiarid grassland: physiological responses to shifts in rain patterns. *Acta Oecologica–International Journal of Ecology, under review.*

CLIMATE CHANGE EFFECTS IN A SEMIARID GRASSLAND: PHYSIOLOGICAL RESPONSES TO SHIFTS IN RAIN PATTERNS

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Abbreviations: Q, rainfall quantity; F, rainfall frequency; *A*, net photosynthesis at leaf-scale; g_s, stomatal conductance at leaf-scale; IWUE, intrinsic water use efficiency; F_v/F_m, maximum photochemical efficiency of photosystem II; PAR, photosynthetically active radiation; LT, leaf temperature; RH, air relative humidity; ITRV, inter-treatment response variability; ISRV, interseasonal response variability; IARV, inter-annual response variability.

Abstract

Changes in precipitation patterns affecting both rain quantity and its temporal distribution have been predicted for the Mediterranean Basin. This three-year study was performed to determine the physiological response of *Macrochloa tenacissima*, a dominant species in the Western Mediterranean grasslands, to these changes. A rainfall manipulation experiment was therefore conducted to test the interaction of two factors: quantity (Q) and frequency (F) of rainfall, both at three levels (100%, 75% and 50% of natural rainfall). A mobile transparent polycarbonate rain-out shelter was designed to cover experimental plots of *M. tenacissima* when it rained, and then treatments were implemented by watering. Reductions in Q and F caused seasonal down-regulation of net photosynthesis (*A*) and stomatal conductance (g_s), but the first variable showed greater resistance to change. At annual scale, only F led *A* to lower rates. The decrease in Q and F had opposite effects on intrinsic water use efficiency (IWUE), enhancing and diminishing it, respectively. However, the response to Q was stronger, even exceeding the range of natural inter-annual variability. No rainfall reduction treatment decreased F_v/F_m regarding ambient treatment. In conclusion, although the responses to the simulated rainfall patterns did not surpass the current seasonal oscillations of *M. tenacissima*'s physiological parameters, it did cause a down-regulation of its gas exchange and increase its water use efficiency.

Keywords: *Macrochloa tenacissima*; *Stipa tenacissima*; gas exchange; intrinsic water use efficiency; F_v/F_m; Mediterranean.

1. Introduction

Although the predicted changes in rainfall are subject to higher uncertainty than temperature (De Castro et al., 2005), climate change models for the Mediterranean region of Europe have found robust signs indicating a decrease in both quantity and number of annual rainfall events. It is also expected changes in their seasonal distribution and size, which will lead to a greater concentration of events in winter, more prolonged droughts in summer and an increase in extreme events (Giorgi & Lionello, 2008). The effects of annual rainfall amount on vegetation have been studied extensively (e.g., Le Houérou & Hoste, 1977; Aronson & Shmida, 1992; Epstein et al., 1997; Knapp & Smith, 2001). However, evidence of the importance of other rainfall pattern components on ecosystem responses to climate, such as seasonal timing, frequency and intensity of precipitation and drought length have increased since the start of the century (e.g., Bates et al., 2006; Chou et al., 2008; Knapp et al., 2008).

Rain manipulation experiments in hyperarid, arid, semiarid and dry-subhumid ecosystems (drylands) have been rarely implemented until recent years (e.g., Yahdjian & Sala, 2006; Thomey et al., 2011; Tielbörger et al., 2014). However, this fact is not an indication of lack of interest, as they occupy ~ 41% of the terrestrial surface, contain ~ 46% of global carbon reserves (Safriel & Adeel, 2005), support ~ 50% of livestock (Puigdefábregas & Pugnaire, 1999), and water availability mediates the responsiveness of these ecosystems to global change (Huxman et al., 2004). Plant community responses to changes in rainfall patterns vary according to the characteristics (such as phenology, morphology and physiology) of the component species (Robertson et al., 2010). *Macrochloa tenacissima* (L.) Kunth (= *Stipa tenacissima* L., alpha grass, esparto) is a rhizomatous, C_3 perennial tussock grass widespread and endemic in Western Mediterranean drylands (Gutiérrez, 2000), and one of the few species which is usually dominant in its community. So exploring how this species responds to changes in rainfall can reflect the direction of the responses at community level. In addition, this species is ecologically important, not only for its spatial predominance, but also due to its morpho-physiological adaptation to this semiarid climate (Ramírez, 2006). Xerophytic communities in Mediterranean grasslands must endure different gradients of water, light and temperature stresses caused by harsh seasonal variations (Madon & Médail, 1997). The *M. tenacissima* strategy for coping with wide variability in abiotic

stress, especially related to water availability, is seasonal changes in its physiological parameters (e.g., Domingo et al., 1991, 2003; Haase et al., 1999; Balaguer et al., 2002; Ramírez et al., 2008). In light of the above, the purposes of this three-year study were to: (i) evaluate *M. tenacissima* plant-scale ecophysiological responses to changes in rainfall patterns (amount and frequency of precipitation); (ii) explore the consistency of these responses on two time scales (seasonal and annual), and any change in their importance; (iii) determine whether the responses to the simulated rainfall patterns could exceed variability in the response to the current rainfall pattern. To pursue these objectives, we designed a full-factorial rainfall exclusion experiment in which two factors were manipulated, rainfall quantity (Q) and frequency (F). Ecophysiological responses of *M. tenacissima* to the treatments were monitored by measuring its leaf-scale gas exchange (CO_2 and H_2O) and maximum PSII photochemical efficiency (F_v/F_m) after each rainfall event for a continuous three-year period.

Previous studies on *M. tenacissima* suggest that a decrease in rainfall will limit its carbon assimilation (Haase et al., 1999), but it can tolerate prolonged drought because it is able to withstand extreme leaf dehydration (Balaguer et al., 2002). In addition, this species can respond quickly to individual water pulses to partly recover gas exchange activity after severe water stress (Pugnaire et al., 1996). These physiological features, as well as apparent coupling of leaf water content and soil moisture, have led to its classification as a functional poikilohydric species (Balaguer et al., 2002). Hence, we hypothesized that: (i) the predicted changes in rainfall patterns cause down-regulation in net photosynthesis (A), stomatal conductance (g_s) and F_v/F_m; (ii) severe water stress increases the instantaneous intrinsic water-use efficiency (IWUE), as in a previous study in which this variable increased sharply with decreasing g_s (Ramírez et al., 2009); (iii) response to change in rainfall could surpass the current inter-annual response range, but this is less likely for interseasonal response since *M. tenacissima* is a drought resistant species with several water control mechanisms, one of them consisting in reducing gas exchange near zero in the season with increased water stress (Haase et al., 1999; Ramírez et al., 2009).

2. Materials and methods

2.1. Study site

Rainfall manipulation was done at the Balsa Blanca experimental site, an *M. tenacissima*-dominated grassland with gentle topography located in the Cabo de Gata Natural Park, Almería, SE Spain (N36°56′26.0″, W2°01′58.8″) at 200 m a.s.l. The climate is semiarid warm Mediterranean, with prolonged summer droughts. Mean annual precipitation and temperature are ~ 220 mm and 18ºC, respectively, with strong intra- and inter-annual variability in precipitation. This rainfall is far from enough to compensate the potential evapotranspiration (~ 1390 mm). The soil at Balsa Blanca is classified as Calcaric Mollic Lithic Leptosols (IUSS Working Group WRB 2006). It is shallow, of variable depth (down to a maximum of ca. 0.3 m, but on average 0.1 m), stony, alkaline (pH > 8) and with carbonate saturation that has led to the formation of a petrocalcic horizon. This horizon is highly permeable due to its porosity and the presence of fissures and fractures (Rey et al., 2012).

The vegetation is dominated by *M. tenacissima*, which represents ~ 80–85% of the cover of vascular plants (Oyonarte, pers. comm.). This ecosystem also includes xeric shrubs (e.g., *Phlomis purpurea* L., *Thymus hyemalis* Lange, *Thymelaea hirsuta* L., *Ulex parviflorus* Pourr.), some other grasses (*Brachypodium retusum* Beauv.) and scattered individuals of climactic shrubs (*Chamaerops humilis* L., *Olea europaea* L. var. *sylvestris* Brot., *Rhamnus lycioides* L.). Annual plants show considerable biodiversity, although cover is usually low. There are no trees, and lichen (*Diploschistes diacapsis* Lumbsch., *Cladonia convoluta* Cout.) or moss-dominated biocrusts often occupy plant interspaces. Vascular plant cover is ~ 55%, and according to Mora & Lázaro (2013), this vegetation has not undergone significant alteration since at least 1955.

2.2. Experimental design, setup and effects on microclimate

A full-factorial rainfall manipulation experiment was setup at Balsa Blanca, to test the Q and F factors in February of 2009, excluding all natural rainfall with an automatic mobile shelter, and performing manual irrigation treatments after each rain event. The reduction in precipitation in the Mediterranean Basin forecasted by climate models has a wide seasonal range of ~ 15%-20% annually, but up to 30%-45% in the driest season

(Bates et al., 2008; Giorgi & Lionello, 2008; Mariotti et al., 2008). We set three levels in each factor (100%, 75% and 50% of the natural rainfall during the experimental period) since use of more than two factor levels along a resource gradient is recommended to test different fitness or ecophysiological response scenarios (Feng & van Kleunen, 2014). Crossing the two factors and three levels made nine treatments, each with three replicates, so 27 *M. tenacissima* tussocks were used in plots shaped and sized to the plants. The replicates of each treatment were applied to a small (< 0.12 m^2), medium (0.12–0.25 m^2) and large (> 0.25 m^2) tussock to include all the size and age variability in the site's natural population (Fig. Supplementary material (S) 1). Uniform distribution of these parameters during the treatments was considered important since previous studies have revealed that small and juvenile tussocks are more vulnerable to water stress and their physiological performance is different from mature tussocks (Armas & Pugnaire, 2005; Ramírez et al., 2008). The plots were carefully selected to fulfill the following criteria: (i) minimal area including 27 variable-sized tussocks; (ii) easy access for construction of the shelter; (iii) very little slope to avoid distortion of irrigation treatments by runoff.

The mobile 40 m^2 transparent polycarbonate shelter with a rain sensor was specially designed to automatically cover the plots when it rained, and uncover them the rest of the time to minimize micrometeorological disturbances (Fig. 1). The shelter was open-sided with a curved roof having a minimum and maximum height of 1.55 and 2.40 m, respectively, to ensure enough air circulation underneath it and thus prevent a greenhouse effect when it was over the plots. Two methacrylate plates were also added on the frontal sides to minimize entry of rain to the rain-out zone. Shelter orientation was north-south to minimize shading on the plots by the structure. Two rain gauges with HOBO dataloggers (Onset Computer Corporation, Bourne, MA, USA) were installed, one outside the shelter and another inside, the latter recording precipitation entering in the rainfall exclusion zone before it was covered by the shelter. All the precipitation that was kept from entering the rainfall exclusion zone was collected by two gutters along the sides of the shelter and stored in two 500 L tanks. This water was used for the rain treatments, which were applied with a sprinkler (to simulate natural rainfall), between the first and third day after each rainfall event.

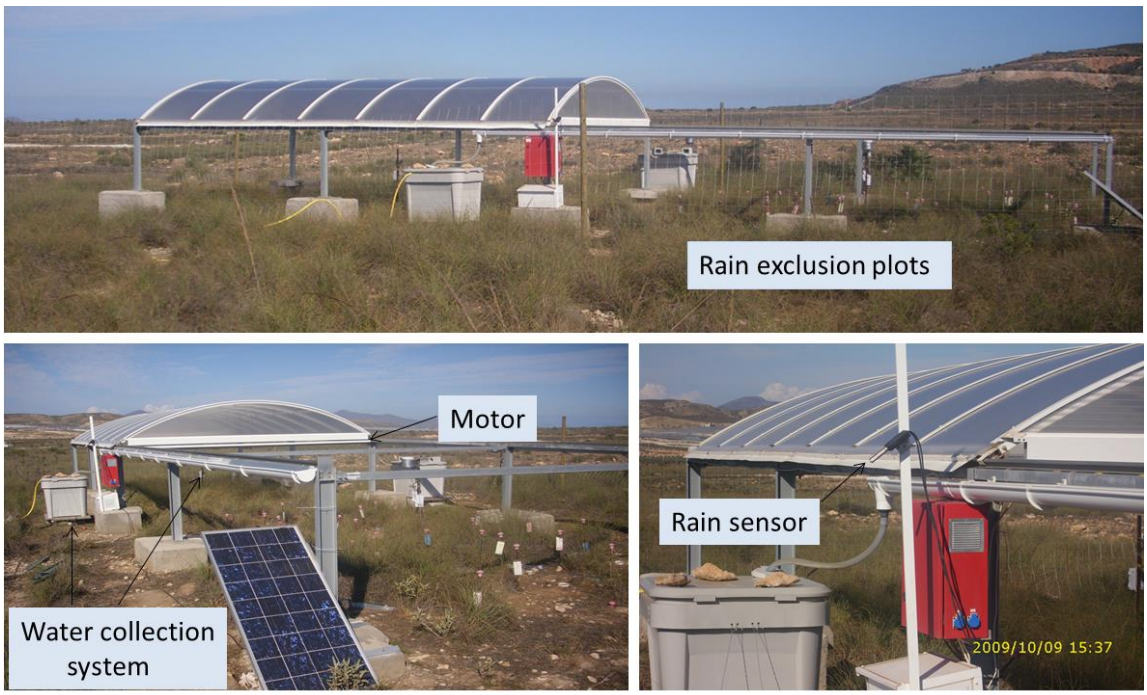

Figure 1. Mobile rain-out shelter showing its most important components.

Two criteria were established for defining an event: (i) rainfall events had to be at least two days apart, the time required to perform treatments and ecophysiological measurements, and (ii) there had to be at least 2 mm of rainfall, because below this threshold canopy interception loss in this species is nearly 100% (Domingo et al., 1998). The amount of water to be used for treatments was calculated as the difference between the two rain gauges, and the mm of rain were converted to ml for watering taking into account the area of each plot. The F factor was run with days without watering treatment and days with compensatory watering to maintain the full amount of Q (see explanation in Table 1).

To avoid water losses or gains unrelated to the treatments, in addition to installing the plots in a place with very little slope (ca. 2–4º), they were delimited by 20-cm-high metal plates driven into the ground as deeply as possible. As a third precaution, the treatments with the least possibility of generating runoff were placed at the top of the slight slope, whereas the treatments that could generate more runoff because of the concentration of rain in fewer events (e.g., Q100-F50) were at the bottom (see Fig. S1). In addition to the shelter treatment plots simulating natural ambient (Q100-F100; 'ambient treatment'), we selected another ten plants (reference plots) outside the rainfall exclusion zone (for logistic reasons, 300 m away from the shelter) encompassing the full range of sizes and ages. In these plants, the same physiological monitoring as those

under the shelter was performed to determine whether the response of the plants with controlled watering was similar to that of plants under natural rainfall.

Table 1 Example of F factor irrigation schedule according to natural rainfall events. White cells: days with irrigation treatment; lined cells: days without irrigation treatment; grey cells: days with compensatory irrigation, i.e., sum of the amount of two consecutive rain events with their corresponding Q factor percentages.

Events	F100	F75	F50
1			
2			Events 1 and 2
3			
4			Events 3 and 4
5		Events 4 and 5	
6			Events 5 and 6
7			
8			Events 7 and 8
9		Events 8 and 9	

To monitor micrometeorological conditions and assess the effects of the shelter on the plots, we measured air and soil temperatures, air relative humidity (RH) and photosynthetically active radiation (PAR) in the rainfall exclusion zone and in the area covered by the shelter when it was not raining. Surface soil temperature (0–0.02 m) and PAR were continuously monitored using RT-1 and QSO-S sensors, respectively (Decagon Devices Inc., Pullman, WA, USA), and air temperature and RH using VP-3 sensors (Decagon Devices Inc.). These data were collected on two Em50 Digital/Analog Dataloggers (Decagon Devices Inc.) and averaged every 5 min. These micrometeorological measurements showed that PAR was the variable most altered by the shelter, causing ~ a 50% reduction on sunny days (Fig. S2a) and ~ 20–30% on cloudy days (Fig. S2b). However, as only covered the plots during rainfall events, the average seasonal reduction was 14%, 6.4%, 1.6% and 6.6% in winter, spring, summer and autumn, respectively. The increase in soil temperature under the shelter was only relevant in the early hours of the afternoon in summer, rising around 8ºC (Fig. S3a),

because this is the season with the highest natural temperatures (Fig. S3b). However, this is the season that the shelter covered less time the experimental plots due to the rainfall regime. As the open sides allowed natural air circulation, the air temperature only rose from 1 to 2ºC under the shelter in all seasons. RH tended to be slightly lower under the shelter, but the difference was only ~ 3%.

2.3. Natural rainfall pattern during the experiment and performance of the treatments

Precipitation during the two hydrological years was above the long-term average of 220 mm. The first hydrological year was exceptionally wet, doubling this average (Fig. 2). This deviation from the mean occurred only once in the long-term data (1961–1990; data source from AEMET, Spanish Agency of Meteorology). The second hydrological year was close to the long-term average, but also surpassed it. Winter was the season with the most rainfall, and summer with the lowest, which is the normal pattern in Mediterranean semiarid ecosystems. Q treatments were close to the annual experimental target (Fig. 2), but with a downward deviation due to some shelter displacement failures. In the F75 and F50 treatments, quantities were concentrated on fewer rainy days, causing events of greater magnitude and intensity (Fig. S4). The distribution of the events also had a maximum in winter and a minimum in summer, with the highest seasonal number of events in winter of 2010, the rainiest period during this experiment, and the lowest in summer of 2011 (Fig. 2).

2.4. Ecophysiological measurements

Ecophysiological measurements of the reference plants were done on the same day that irrigation treatments took place. After watering, we waited the same time that had elapsed between the rainfall event and data collection on the reference plants to perform the measurements on the treated plants. This elapsed time regarding watering (from one to three days later) is when the response to water pulses is maximal in *M. tenacissima* (Pugnaire et al., 1996). In both cases (shelter internal and external plants), the measurements were taken at the time of highest photosynthetic activity of the day, which was estimated according to previous full-day cycle measurements in different seasons (unpublished data). Due to the high uncertainty of being able to correctly

place humidity sensors in a shallow soil with high presence of stone fragments, and the possibility of causing damages in the roots, soil water content was not recorded in the plots. Therefore, this measurement schedule was considered the appropriate to relate ecophysiological responses with accurate irrigation data and to better discern the different responses to treatments in a functional poikilohydric species.

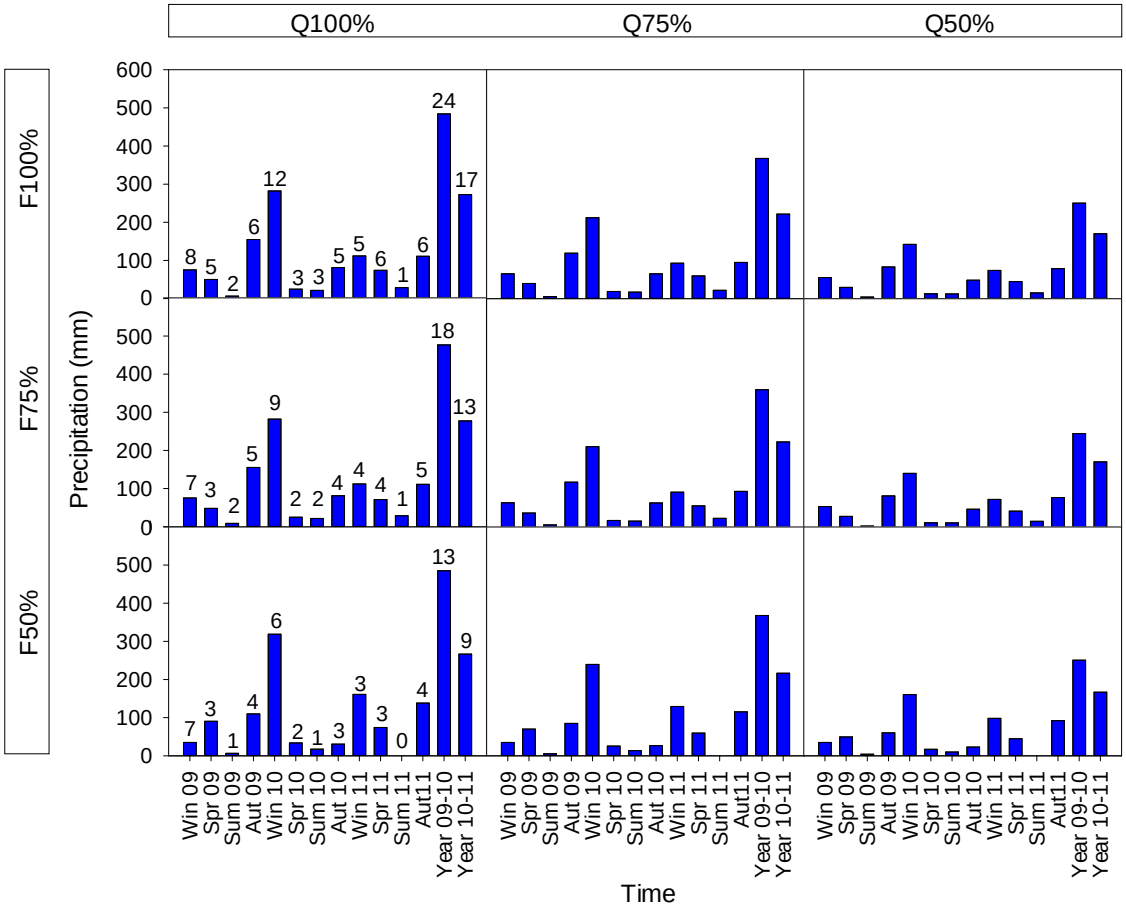

Figure 2. Irrigated water (mm) per treatment and time interval (seasons and hydrological years). The numbers above the bars in the left section denote the number of rainfall events per frequency level and time interval.

Leaf gas exchange measurements were performed using an infrared gas analyzer (LI-6400; LI-COR, Lincoln, NE, USA) coupled to a chamber with an area of 6 cm^2 and an LED light source (model 6400-02B, LI-COR). CO_2 and H_2O zeros and flow meters were calibrated before each data collection. Natural input air was taken at two meters above the soil surface by a tube and a 6-L bottle was used as a buffer volume before the air entered into the system. Prior to measurement in each day, PAR and block chamber temperature were set to standardize the environmental conditions during data collection.

PAR corresponded to a sunny day and the chamber temperature was the natural air temperature at start of measurement (dataset range: 800–2000 μmol m^{-2} s^{-1} in PAR; 17–30ºC in temperature). System airflow was regulated according to the photosynthetic activity, with a minimum of 200 μmol s^{-1} at very low activity (near compensation point), and a maximum of 350 μmol s^{-1} at high activity, and was also fixed at the beginning of the measurements of each day. During the measurements, sample and reference infrared analyzers were matched about every 15 min. With these records, three ecophysiological variables were acquired: *A*, g_s and IWUE. *A* and g_s rates were estimated with a leaf area correction, calculated as a flat surface when the leaf was not rolled up, and as a semi-cylinder when the leaf rolled up due to water stress. In each plot, from four to six leaves were placed in the chamber, all facing north to reduce the PAR variability incident on the samples prior to their introduction into the chamber. Only mature leaves were used because they represent the largest area with photosynthetic activity all year around (78–100% of plant green area) and they usually have a slightly higher photosynthetic rate than young leaves (Haase et al., 1999). The measurements were performed in the longitudinal middle area of the leaves to reduce the within-leaf gas exchange variability (Ocheltree et al., 2013). IWUE was calculated as a ratio of A/g_s.

After gas exchange measurements, F_v/F_m was recorded on the same leaves with a Handy PEA fluorometer (Hansatech, Kings Lynn, UK). Dark-adapted F_v/F_m reflects the potential quantum efficiency of PSII and has been used extensively as a sensitive indicator of plant photosynthetic performance (Maxwell & Johnson, 2000). Therefore, prior to measurement, the leaves were adapted to the dark with leaf clips (Hansatech, UK) for 30 min. Measurements using a saturating light pulse of 3000 μmol m^{-2} s^{-1} took 5 s. Before measuring, autogain was set to maximise the signal during measurement to ensure that the samples did not exceed the instrument scale (Hansatech Instruments, 2006).

2.5. Data analyses

Prior to the analysis, seasonal and annual averages were calculated for each variable and plot using the dataset of event measurements. Annual analyses were performed by hydrological years (from September to August of the following year), since this represents both the natural cycles of rainfall and plant species activity. The analyses of

the four physiological variables (*A*, g_s, IWUE and F_v/F_m) were implemented in InfoStat (ver. 2013p, http://www.infostat.com.ar.), a user-friendly interface for R (ver. 2.15.2). General linear mixed models (LMMs) with repeated measures were used to test seasonal and annual differences in the physiological variables among the treatments.

Q, F, Time (season or year) and their interactions were included in the models as fixed factors, and the plots were entered as a random factor on the intercept to reduce the effect of intraspecific variability of the responses to the treatments. The effects of entering leaf temperature (LT) in the *A* models, and PAR in the *A*, g_s and IWUE models as covariates were also tested. These covariates were recorded by the LI-6400 system at the same time as gas exchange measurements, and were also entered in the model as seasonal or annual averages. Only when the covariates had a significant effect at $p < 0.05$ were included in the models, and if both of them were selected in the case of the *A* models, possible multicollinearity between them was analyzed using the variance inflation factor (VIF) parameter. Analysis of annual F_v/F_m required rank transformation of the data for a normal distribution. In each model selected, Fisher's least significant difference (LSD) post-hoc test was applied to the most complex interactions of the factors showing significant differences for all pairwise comparisons. This procedure was also performed to analyze and compare the physiological activities of the ambient treatment and reference plots.

Inter-treatment response variability (ITRV) was analyzed in the four physiological variables and compared to the natural interseasonal and inter-annual response variability (ISRV and IARV, respectively) to the current climate ambient treatment. ITRV was estimated as the mean rate of change (calculated for each plot size) between the ambient treatment and the treatments with the strongest response (i.e., the treatments with the maximum or minimum mean in each period analyzed). ISRV and IARV were estimated as the mean ambient treatment rate of change (also calculated separately in the three plots) between seasons and years, respectively. *T* tests were performed to determine whether the ITRVs were significantly different from the ISRV and IARV. When the variances were not homogeneous, power transformations were used.

3. Results

3.1. *ISRV and IARV of M. tenacissima under the ambient rainfall pattern*

In gas exchange variables, ISRV was greater than ITRV, as ambient treatment had rates of change between the seasons with maximum and minimum *A* and g_s of 79.60% and 79.92%, respectively, in 2009-10, increasing to 95.01% and 85.85% in 2010-11 (Table Appendix 1). ISRV rates in F_v/F_m were lower and more constant in the two hydrological years, 22.79% and 22.35% in the first and second year, respectively, whereas the most different ISRV rates between these two years were found in IWUE (20.04% in 2009-10, and 68.51% in 2010-11). The difference in rainfall between the two years is reflected in the average annual gas exchange, as in the first rainy year *A* and g_s were higher (Table Appendix 2). However, despite these two very contrasting hydrological years, the IARV was four to five-fold lower than the ISRV. The IARV was negligible in IWUE and F_v/F_m.

3.2. M. tenacissima response to the rainfall reduction treatments

The main findings related to ecophysiological responses to changes in rainfall patterns are: (i) g_s and IWUE were more sensitive to change than *A* at seasonal scale; (ii) F factor played a more important role than Q factor in gas exchange shifts; and (iii) no rainfall reduction treatment caused a down-regulation of F_v/F_m regarding ambient treatment. In the next sections we described the most complex interactions for each variable.

3.2.1. Net photosynthesis (A)

For *A*, on a seasonal basis, Q × F × Time interaction was found statistically significant (Table Supplementary material (S) 1). The significant effect of the interaction took place in winter and autumn of the second hydrological year, in 2011 (Fig. 3a). In both seasons, Q100-F100 had the highest *A*, but in winter only Q75-F75 was significantly lower than the ambient treatment, whereas in autumn there were four treatments with a significant decline in *A* (Q100-F75; Q75-F75; Q75-F50; Q50-F50). In this first season, Q50-F100 and Q75-F50 also had significantly higher *A* rates than Q75-F75, so

there was no linear relationship between factor reduction and *A* response. In the analysis of annual averages, only F and Time factors caused effects on this variable without interacting between them (Table S1). Reduction in F negatively affected the performance of *A* at 75 and 50 levels. Hydrological Year 2009-10 had higher average *A* than 2010-11, most likely as a result of higher rainfall in the first year (Fig. 3b).

3.2.2. Stomatal conductance (g_s)

For g_s, Q × Time and F × Time interactions were found statistically significant in the seasonal analysis (Table S1). The F factor had more seasonal impact on this variable than the Q factor (Fig. 3c) or the interaction of the three factors on *A* variable. Taking into account both factors, there were six periods with significant responses to the treatments. The ambient treatment had higher g_s than the 75% and 50% reductions in two and three periods, respectively. But in autumn of 2010, the 75% reduction was the level in F with a greater g_s than the 50% reduction. No significant effect of Q or F was found in the analysis of hydrological years (Table S1). As in *A*, Hydrological Year 2009-2010 had higher average g_s than in the following year (Fig. 3d).

3.2.3. Intrinsic water use efficiency (IWUE)

Seasonal Q × Time interaction and F as main effect were found statistically significant (Table S1). In the year 2009, IWUE at Q75 was significantly higher than at Q100, but in year 2011, the Q50 reduction level showed the best IWUE performance (Fig. 3e). Winter was the only season without any significant effect, and after the rainy winter of 2010, no treatment had any effect in any season until spring of 2011. In the F factor, IWUE was significantly lower at F50 than the other levels (Fig. 3e). As on the seasonal scale, the Q factor led to significant annual differences through its interaction with Time, and the F factor was statistically significant without any interaction (Table S1). The Q factor only had an effect in the last year, when the IWUE increased in both Q reduction levels. In the F factor, the post-hoc result was the same as at seasonal scale, with a lower IWUE at F50 than the other levels (Fig. 3f). Therefore, IWUE was sensitive to changes in intra-annual Q and F close to the long-term mean annual amount of precipitation, but only to F when the ambient amount of precipitation doubled the long-term mean.

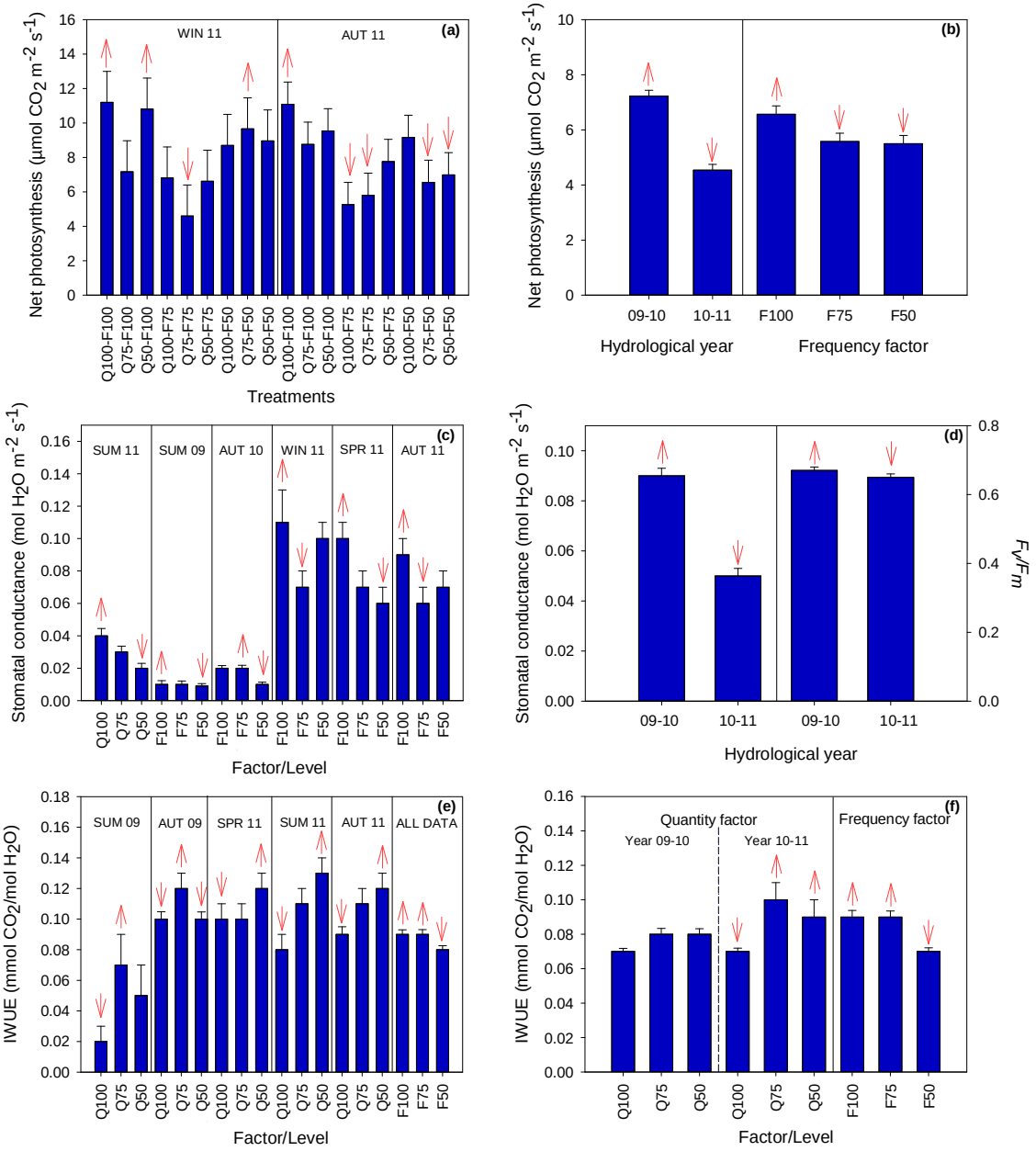

Figure 3. Fisher's least significant difference (LSD) post hoc results for seasonal and annual *A* **(a**, **b)**, g_s **(c**, **d)** and IWUE **(e**, **f)**. F_v/F_m means **(d)** show the results on an annual scale. The most complex significant interactions of each factor in these variables and temporal scales are shown in the periods that took place. The main significant effects of the factors are shown when they did not have any significant interaction. Data are mean ± SE (n = 3). Upward arrows indicate significantly higher means than those with downward arrows.

3.2.4. Maximum photochemical efficiency of PSII (F_v/F_m)

As in variable *A*, a seasonal Q × F × Time interaction was found in F_v/F_m (Table S1). In the first measurement season (spring of 2009), the mean F_v/F_m with Q50-F75 irrigation was significantly higher than the means in all treatments with Q100 level

and in Q50-F50 (Fig. 4). Q50-F75 also had a significantly higher F_v/F_m than for Q75-F75 in autumn of 2011. In summer of 2010, the mean of F_v/F_m in Q50-F50 was significantly lower than in the rest of the treatments, except in those with Q100 water application. As in *A* and g_s variables, there was a significant difference between hydrological years in the Time factor, with no interaction with other factors (Table S1; Fig. 3d). Neither Q nor F factors caused any response in the main effects or interactions at annual scale.

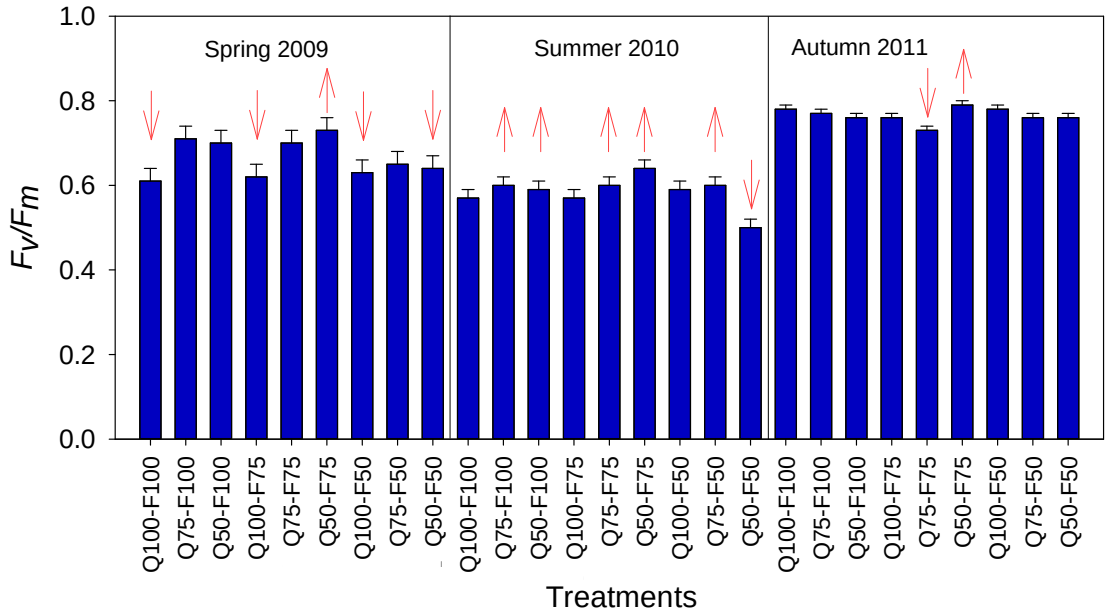

Figure 4 Fisher's least significant difference (LSD) post hoc seasonal-scale results for F_v/F_m. The Q × F × Time interaction is shown only with the period when it took place. Data are mean ± SE (n = 3). Upward arrows indicate significantly higher means than those with downward arrows.

3.3. Comparison of ambient treatment and reference plots

Seasonal physiological activity was similar in both ambient treatment and reference plots most of the time. However, in *A* and IWUE, there were three periods when statistical differences between these plots were detected, with an interaction between the Time and Plot-type factors (Table S2): Summer of 2009, winter of 2010 and autumn of 2011 in *A*, and summer of 2009 and autumns of 2010 and 2011 in IWUE (Fig. S5a, c). In all these periods, except IWUE in autumn of 2011, the values of the reference plots were higher than for the ambient treatment plots. Differences in g_s were found only in winter of 2010 and summer of 2011. In the first case, g_s was

higher in the reference plots, and in the second, in the ambient treatment plots (Fig. S5b). Although the results of the mixed model indicated a Plot-type × Time interaction in F_v/F_m (Table S2), significant seasonal differences these plots were not found in the post-hoc analysis (Fig. S5d).

The analysis of means for hydrological years showed significant differences in *A* between years, but not between the types of plots (Table S2; Fig. S6). In g_s the interaction Plot-type × Time was almost significant (Table S2), and a post-hoc analysis was performed because is considered advisable when the F value is over 2 and the sample size is small (Fry et al., 2013). This analysis showed that the reference plots in 2009-10 had significantly higher mean g_s than the rest of the means (Fig. S6). In conclusion, the differences between ambient and reference plots detected in some periods could not be due to micrometeorology alterations by the shelter since it was over the plots only ~ 1% of the experimental time, but due to spatial variability of the physiological responses driven by different factors that determine water availability at plot scale.

3.4. Comparison of M. tenacissima ITRV with its ISRV and IARV

The *A* and g_s ITRV rates calculated by comparing the ambient treatment and treatments with the most down-regulation in each season were significantly lower than the ambient treatment rates of change between seasons in most periods (Table Appendix 1). ITRV rates were calculated using the treatments with the highest values in IWUE and F_v/F_m variables, and in this case only two and four periods, respectively, had significantly greater ISRV (ITRV represented in column four, and ISRV in columns five and six of Table Appendix 1). However, annual ITRV was significantly higher than the IARV in IWUE (columns four and five, respectively, of Table Appendix 2). This occurred in both hydrological years, although the strongest change in IWUE from a rainfall reduction treatment was in the second year. In this variable, the rate of change of the treatment causing the minimums was also calculated, as the F factor caused down-regulation. In this case, the biannual means were used since F did not interact with time. This rate of change was very close to the IARV rate, as was also the case for F_v/F_m. ITRV in *A* and g_s doubled the IARV rate in the second hydrological year, but this difference was not statistically significant.

4. Discussion

4.1. Intra-seasonal and intra-annual effects of the simulated rainfall patterns

Unexpectedly, Q50-F75 was the treatment with the highest F_v/F_m. This treatment also had significantly highest values of NDVI parameter (data not show) than the rest of the treatments. These two variables are sensitive to chlorophyll content, especially in the low range of values and under drought conditions (Gitelson & Merzlyak, 1997; Guo et al., 2008). Hence, the most plausible explanation for this result is that plants that of this treatment had at the beginning of the experiment higher average chlorophyll content due to interindividual natural variability (see Fig. 4, where in the first season F_v/F_m of Q50-F75 is significantly higher than several treatments). During the experimental period these differences were diminishing, but in moments of increased drought, when chlorophyll contents are minimal, its influenced over F_v/F_m was reflected again in lesser magnitude (F_v/F_m of Q50F75 was only significantly higher than one treatment in two periods of drought) due to the higher sensitivity of this parameter under this conditions. In conclusion, no rainfall reduction treatment adversely affected F_v/F_m parameter, pointing out the great resistance of *M. tenacissima* photosynthetic apparatus to water stress.

Performance of *A* in this species showed resistance to change in response to intraseasonal rainfall availability, as until winter of 2011 the treatments did not cause any effect (Fig. 3a). However, this resistance was lower than in shrub species in this region according to Miranda et al. (2011), who only found significant gas exchange response after four years of 30% reduction in water and seasonal variation in precipitation, and significant differences between treatments disappeared in the extremely rainy Hydrological Year 2009-10. The fact that our experiment was also performed during this period probably facilitated this seasonal resistance to change in *A* rate. Most Mediterranean herbaceous species are able to maintain their physiological performance in an environment of irregular precipitation by using several water use efficiency control strategies (Moreno et al., 2008). Stomatal behaviour is the main mechanism plants have for controlling the trade-off between water loss and carbon gain, and their closure is among the first physiological events occurring in response to a decrease in water availability (Flexas et al., 2004). We thus

found a g_s response to the treatments from the first experimental year, which was also the driver for rapid changes in IWUE (Fig. 3c, e).

In line with studies that highlight the role of rainfall frequency behind shifts in ecosystem structure and functionality (e.g., Knapp et al., 2002; Büdel et al., 2009; Heisler-White et al., 2009), in our experiments, the F factor caused the greatest responses in the gas exchange variables (*A* and g_s). In *A*, a seasonal interpretation is more complex due to the three-term interaction, Q × F × Time. Supporting our initial hypothesis, one and four rainfall reduction treatments caused down-regulation of *A* in winter and autumn of 2011, respectively (Fig. 3a). However, in the winter of 2011, Q75-F50 had greater *A* rates than Q75-F75. Previous studies have found that more intense events, such as the one caused by our F reduction in irrigation, infiltrate more deeply into the soil (e.g., Weltzin et al., 2003; Loik et al., 2004; Knapp et al., 2008). This effect can benefit water availability in drylands, but in contrast, a decrease in F also causes an increase of days between events. Thus, depending on the number of events, their sizes, and their temporal distribution, there is a threshold where F no longer favours water availability but limits it instead.

In winter of 2011, the F50 distribution caused two big events (> 20 mm) to be combined in one, whereas in F75, a big event combined with a small (≈ 5 mm) one, so rain intensification was negligible (see Fig. S4) and the negative effect of increased days between events prevailed. This positive effect of event concentration probably only affected *A* in winter because the soil porosity is higher (Mora & Lázaro, 2014) and the evaporative demand is lower (Serrano-Ortiz et al., 2014). But in autumn of 2011, Q mediated, causing a threshold in the negative effect of F50, with down-regulation at Q75-F50 and Q50-F50, but without any significant response at Q100-F50. On the other hand, F also mediated the response to Q, as in the F reduction treatments *A* suffered a decrease even maintaining the ambient Q. Q50-F75 was unaffected probably, as pointed out above, because of the effect of a greater chlorophyll concentration in the samples of this treatment.

As expected, g_s was down-regulated by reduction in both Q and F, but especially F (Fig. 3c). In some periods (winter and autumn of 2011), the most stomatal closure was at Level F75, but in others (summer of 2009, autumn of 2010 and spring and summer of 2011) it was at F50. As in *A*, these responses suggest an effect of F on water availability which is complex and nonlinear. IWUE increased

with the reduction in Q (Fig. 3e, f), because drought-induced stomatal closure restricts water loss more than CO_2 uptake (Ramírez et al., 2009; Jongen et al., 2011). However, Ramírez (2006) reported decreasing IWUE in *M. tenacissima* when soil water content fell below 5%, and even it becomes negative if respiration surpasses photosynthesis. This is consistent with our results, since in the driest periods (summer 09 and autumn 10) *A* was near the compensation point and ambient treatment IWUE was at a minimum (Table Appendix 1). The F50 level triggered a decline in IWUE, which is in agreement with the results of Fay et al. (2011) in a mesic ecosystem, as they suggest that most ecosystem process rate reductions due to increased variability in rainfall is an indicator of lower ecosystem rainfall use efficiency. However, this conclusion cannot be generalized, because in another Mediterranean-type ecosystem with a higher rainfall regime than our study area, water-use efficiency is more related to temperature (Jongen et al., 2014).

The timing and amount of the first rainfalls ending drought are important attributes of Mediterranean precipitation patterns (Swarbreck et al., 2011), as they are critical to triggering biological activity (Beatley, 1974). This fact has been highlighted in the present study, since it determined the effects of the treatment effects in autumn. The results demonstrated that an extraordinarily large event of 85 mm at the beginning of the autumn of 2009 buffered the deleterious effect of F reduction in *A*, and only IWUE had a significant response to the treatments increasing the rate in Q75 level. In autumn of 2010, the situation was the contrary, as there was no rainfall event over 10 mm until the end of the season. So the range of *A* was similar within all the treatments during this period, because of the absence of any relevant rainfall to trigger recovery. However, if the first event after summer is > 25 mm, as was the case in the third autumn, all variables responded to the treatments, showing that the recovery activity period is particularly sensitive to changing rainfall patterns.

4.2. Will the physiological responses to future rainfall patterns exceed their current range of interseasonal and inter-annual variability?

The knowledge of the natural variation of a system is a necessary step in understanding ecological processes and the implications of ecological changes (Landres et al., 1999). One of the goals of this study was to determine whether the shifts in rainfall patterns forecasted for the Mediterranean Basin are a force of change

powerful enough to drive its terrestrial ecosystems beyond their current range of variability. Our results show that, although Q and F had significant seasonal effects on all variables, the main driver of gas exchange activity for this species was the characteristically strong seasonal shifts in precipitation and temperature of the Mediterranean climate, rather than the simulated rainfall patterns.

The Mediterranean climate is characterized by strong seasonal distribution of precipitation, with most of the rainfall concentrated in autumn and winter. Therefore, the warmest months coincide with low rainfall, and *M. tenacissima* adapts its metabolic activity drastically to the rising gradient of environmental stress (water deficit, high temperature and irradiance) from mid-spring to early autumn with down-regulation of gas exchange. Consequently, the course of time over the seasons caused greater changes in the physiological variables (especially in gas exchange rates) than the effects of treatments within each season (Table Appendix 1). However, it must be remembered that the experimental period did not cover the effects of the treatments in an extremely dry year, and therefore we cannot extrapolate the findings of our study to this precipitation range.

Unlike the seasonal scale, we found that ITRV was a greater force of change than IARV in all variables except F_v/F_m (Table Appendix 2). This demonstrates that intra-annual variations in individual event size and the interval between the events influence plant processes regulating carbon and water cycling more than the average annual amount of precipitation (Schwinning & Sala, 2004; Fay et al., 2008). In spite of the wide average differences between ITRV and IARV, the statistical results were not significant in gas exchange variables because there were fewer changes in g_s and *A* in small and medium-size plots, respectively. Therefore, resistance to change in the gas exchange variables due to the simulated rainfall patterns was plant-size specific. In IWUE, the rate of change caused by the treatment with the highest value was out of the range of the IARV, but this did not happen in the treatment with the lowest value. This shows that, although IWUE had an opposite response to F and Q, this variable is more sensitive to change in Q and its annual range will be shifted upward. However, intra-annual response variability in this variable was significantly greater than inter-annual in both years, indicating that IWUE was also sensitive to the distribution of the water inputs along the year.

Another finding is that the magnitude of the change in response between treatments differed in these hydrological years of contrasting amounts of rain. Thomey et al. (2011) also found a differential inter-annual strength of ANPP response to intra-annual Q and F rainfall changes in desert grasslands. Thus, our results support the idea of Thomey et al. (2011) that it is not only important to predict relative changes within years, but also patterns of responses between years.

5. Conclusions

The impact of potential changes in rainfall patterns will depend not only on the predicted scenarios of change, but on the way the ecosystem's dominant species have adapted under current climate conditions (Hanson & Weltzin, 2000). For example, the physiological parameters of Mediterranean woody and annual species have shown great resistance to change under new rainfall pattern scenarios (Miranda et al., 2011; Jongen et al., 2013). In *M. tenacissima*, *A* only had significantly different responses to the treatments in WIN11 and AUT11, and g_s was primarily decreased in the last experimental year, consequently increasing IWUE. Thus, the existence of water use efficiency control mechanisms in this species was responsible of the resistance response to change *A* under different rainfall patterns.

The effects of the treatments observed in this experiment were mediated by the current seasonal rainfall distribution, as the recurrent interaction of the Q and F factors with Time implies (Table S1). These effects were complex in the gas exchange variables, probably because there were no linear relationships between the factor levels and their interaction with water availability. For example, under some circumstances F75 had a stronger negative seasonal impact on gas exchange than F50, but neither of them increased the rates over the current ambient rainfall pattern. In addition, in spite of the resistance to change detected at seasonal scale, both levels of frequency reduction caused an annual decrease in the *A* rate. Therefore, regardless of the magnitude of change in the factors tested, down-regulation of the gas exchange was detected, a point that must be taken into account in the quantification of carbon and water balances in those areas where *M. tenacissima* is dominant, as well as its influence on novel dynamic climate models.

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APPENDIX

Table A.1 Comparison of the response variability caused by the reduction treatments and the natural interseasonal response variability of the ambient treatment.

Season	Ambient treatment	Treatment with maximum effect	% change treatment	% change with previous season	% change Max./min. season
		A(µmol CO_2 m^{-2} s^{-1})			
SPR 09	9.56	Q50-F100 / 6.56	21.67%	-	-
SUM 09	0.24	Q100-F50 / 0.15	56.67%	**97.67%**	-
AUT 09	6.48	Q75-F50 / 2.98	53.00%	93.04%	49.99% / 51.18%
WIN 10	9.56	Q75-F75 / 7.41	20.33%	30.89%	30.26% / **70.66%**
SPR 10	13.61	Q50-F100 / 12.63	22.33%	30.26%	0.00% / **79.60%**
SUM 10	2.74	Q50-F50 / 1.87	28.67%	**79.60%**	**79.60%** / 0.00%
AUT 10	0.64	Q100-F50 / 0.22	39.82%	78.86%	**95.01%** / 0.00%
*WIN 11	11.2	Q75-F75 / 4.60	49.70%	**95.01%**	0.00% / **95.01%**
SPR11	10.77	Q75-F50 / 5.51	40.84%	40.18%	40.18% / **93.63%**
SUM 11	4.58	Q50-F50 / 2.15	53.28%	46.23%	54.90% / **87.01%**
*AUT 11	11.09	Q100-F75 / 5.26	50.63%	58.09%	-
		g_s (mol H_2O m^{-2} s^{-1})			
SPR 09	0.10	Q50-F100 / 0.07	28.01%	-	-
*SUM 09	0.012	Q100-F50 / 0.011	65.39%	85.78%	-
AUT 09	0.07	Q75-F50 / 0.03	43.14%	76.23%	59.08% / 38.92%
WIN 10	0.12	Q75-F75 / 0.09	26.52%	36.31%	31.12% / **69.78%**
SPR 10	0.18	Q75-F100 / 0.15	14.78%	31.12%	0.00% / **79.92%**
SUM 10	0.04	Q100-F75 / 0.03	14.07%	**79.92%**	**79.92%** / 0.00%
*AUT 10	0.02	Q75-F100 / 0.01	33.33%	45.26%	**85.85%** / 0.00%
*WIN 11	0.14	Q75-F75 / 0.05	58.44%	85.85%	0.00% / 85.85%
*SPR11	0.13	Q75-F50 / 0.05	59.58%	34.15%	34.15% / **84.17%**
*SUM 11	0.05	Q50-F75 / 0.02	54.65%	59.65%	60.45% / 61.24%
*AUT 11	0.12	Q75-F75 / 0.06	48.89%	52.92%	-
		IWUE (mmol CO_2/mol H_2O)			
SPR 09	0.08	Q100-F50 / 0.10	11.46%	-	-
*SUM 09	0.03	Q75-F75 / 0.04	81.33%	71.69%	-
*AUT 09	0.09	Q75-F75 / 0.13	28.30%	**73.01%**	0.00% / 20.04%

WIN 10	0.080	Q75-F75 / 0.083	12.59%	13.26%	13.26% / 13.51%
SPR 10	0.08	Q75-F75 / 0.09	16.07%	8.63%	15.56% / 9.45%
SUM 10	0.07	Q50-F75 / 0.10	23.61%	9.45%	20.04% / 0.00%
AUT 10	0.03	Q75-F100 / 0.08	50.96%	60.49%	68.51% / 0.00%
WIN 11	0.08	Q50-F50 / 0.11	27.05%	**62.31%**	15.54% / **62.31%**
*SPR11	0.08	Q50-F75 / 0.13	39.50%	23.98%	21.11% / 58.81%
*SUM 11	0.09	Q75-F100 / 0.15	29.92%	21.11%	0.00% / 68.51%
*AUT 11	0.10	Q50-F75 / 0.12	15.57%	14.68%	-
			F_v/F_m		
*SPR 09	0.61	Q50-F75 / 0.73	15.81%	-	-
SUM 09	0.50	Q75-F75 / 0.54	18.59%	17.54%	-
AUT 09	0.73	Q75-F100 / 0.74	3.57%	**30.63%**	3.41% / **21.73%**
WIN 10	0.72	Q50-F50 / 0.74	3.91%	1.49%	2.47% / **20.96%**
SPR 10	0.74	Q50-F75 / 0.75	3.30%	2.47%	0.00% / **22.79%**
*SUM 10	0.57	Q50-F75 / 0.64	10.94%	22.79%	22.79% / 0.00%
AUT 10	0.55	Q100-F75 / 0.59	13.29%	10.08%	22.35% / 0.00%
WIN 11	0.71	Q100-F50 / 0.74	4.37%	22.35%	0.00% / 22.35%
SPR11	0.68	Q75-F100 / 0.71	13.93%	6.11%	6.11% / 18.87%
SUM 11	0.67	Q75-F100 / 0.69	3.81%	10.59%	6.46% / 20.75%
*AUT 11	0.78	Q50-F75 / 0.79	2.93%	**13.62%**	-

* denotes seasons with significant responses to the treatments in the LMM models. Treatments with maximum effect are those that caused minimum seasonal *A* and g_s, and maximum seasonal IWUE and F_v/F_m. The fourth column gives the rate of change from the ambient treatment to treatment with the maximum seasonal effect. The fifth column gives the rate of change between the ambient treatment and its value in the antecedent season. The sixth column gives the rate of change from the ambient treatment to its seasonal maximum and minimum within hydrological years. The seasonal rates of change of the ambient treatment are highlighted in black bold when are significantly higher (p values < 0.05) than the intraseasonal rates of change caused by the rainfall reduction treatments (n = 3). Note that the % of change were not calculated with the values of columns two and three, but as the averages of the % of change per plot-size.

Table A.2 Comparison of the response variability caused by the reduction treatments and the natural interannual response variability of the ambient treatment.

Years	Ambient treatment	Treatment with maximum effect	% change treatments	% change between years
		A(µmol CO_2 m^{-2} s^{-1})		
09-10	7.51	Q75-F75 / 6.50	12.83%	19.89%
10-11	6.21	Q75-F75 / 3.62	40.30%	19.89%
		g_s (mol H_2O m^{-2} s^{-1})		
09-10	0.10	Q75-F75 / 0.07	21.12%	20.51%
10-11	0.08	Q50-F75 / 0.04	45.15%	20.51%
		IWUE (mmol CO_2/mol H_2O)		
09-10	0.073	Q75-F75 / 0.09	**17.40%**	**4.02%**
*10-11	0.071	Q50-F75 / 0.11	**31.91%**	**4.02%**
*Both years	0.072	Q100-F50 / 0.070	3.93%	4.02%
		F_v/F_m		
09-10	0.672	Q50-F75 / 0.70	3.41%	2.50%
10-11	0.667	Q75-F100 /0.668	2.49%	2.50%

* denotes hydrological years with significant responses to the treatments in the LMM models. Treatments with maximum effect are those that caused minimum annual *A* and g_s, and maximum annual IWUE and F_v/F_m. "Both years" in IWUE refers to the treatment with the minimum biannual average. The fourth column gives the rate of change from the ambient treatment to the treatment with the maximum annual effect. The fifth column gives the rate of change from the ambient treatment to its value in the other year. The inter-annual rates of change of the ambient treatment are highlighted in red bold when are significantly higher (p values < 0.05) than the intra-annual rates of change caused by the rainfall reduction treatments (n = 3). Note that the % of change were not calculated with the values of columns two and three, but as the averages of the % of change per plot-size.

SUPPLEMENTARY MATERIAL

Climate change effects in a semiarid Mediterranean grassland: seasonal and annual physiological responses to shifts in rain patterns

Journal: Acta Oecologica–International Journal of Ecology

M. Ladrón de Guevara, R. Lázaro, E. Arnau-Rosalén, F. Domingo, I. Molina-Sanchis and J.L. Mora

Table S1 Results of the MIXED model analysis evaluating treatment effects on *Macrochloa tenacissima* physiological variables: *A* = net photosynthesis; g_s = stomatal conductance; IWUE = intrinsic water use efficiency; *Fv/Fm* = maximum photochemical efficiency of PSII. Factors and covariates are: Q = quantity factor; F = frequency factor. P values below 0.05 are in bold, from 0.05 to 0.10 are in italics.

Factors and covariates	**Seasonal analysis**							
	A		g_s		IWUE		*Fv/Fm*	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Q	0.36	0.702	4.98	**0.019**	12.20	**<0.001**	0.27	0.768
F	2.75	*0.091*	4.01	**0.036**	4.40	**0.028**	0.83	0.454
Time	111.70	**<0.001**	180.35	**<0.001**	27.14	**<0.001**	215.84	**<0.001**
Q × F	0.38	0.821	1.21	0.342	1.21	0.341	0.55	0.701
Q × Time	2.70	**<0.001**	1.68	**0.040**	2.26	**0.003**	3.33	**<0.001**
F × Time	5.79	**<0.001**	1.71	**0.035**	1.43	0.114	2.96	**<0.001**
Q × F × Time	2.73	**<0.001**	0.88	0.679	1.07	0.378	1.88	**0.003**
PAR	48.40	**<0.001**						
Analysis of hydrological years								
Q	0.82	0.457	2.15	0.145	11.91	**0.001**	0.51	0.612
F	3.88	**0.040**	1.77	0.198	5.99	**0.010**	0.47	0.631
Time	127.29	**<0.001**	160.43	**<0.001**	15.29	**0.001**	6.19	**0.023**
Q × F	0.34	0.846	0.39	0.812	1.10	0.387	0.49	0.745
Q × Time	0.06	0.943	0.66	0.530	4.86	**0.021**	1.26	0.308
F × Time	1.92	0.176	1.01	0.382	0.54	0.592	0.22	0.804
Q × F × Time	0.89	0.491	1.37	0.285	2.67	*0.066*	0.13	0.969

Table S2 Results of the MIXED model analysis evaluating the differences of *Macrochloa tenacissima* physiological variables between the ambient and the reference plots (Plot-type factor). *A* = net photosynthesis; g_s = stomatal conductance; IWUE = intrinsic water use efficiency; *Fv/Fm* = maximum photochemical efficiency of PSII. P values below 0.05 are in bold, from 0.05 to 0.10 are in italics.

Factors	**Seasonal analysis**							
	A		g_s		IWUE		*Fv/Fm*	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Plot-type	0.44	0.521	1.84	0.202	2.72	**0.005**	0.00	0.956
Time	94.49	**<0.001**	73.58	**<0.001**	1.63	0.228	39.11	**<0.001**
Plot-type × Time	2.78	**0.004**	2.84	**0.004**	5.74	**<0.001**	4.12	**<0.001**
Analysis of hydrological years								
Plot-type	2.97	0.113	2.57	0.137	1.23	0.291	0.00	0.966
Time	25.03	**0.000**	21.23	**0.001**	0.84	0.380	0.55	0.473
Plot-type × Time	3.61	*0.084*	4.52	*0.057*	2.25	0.162	0.21	0.654

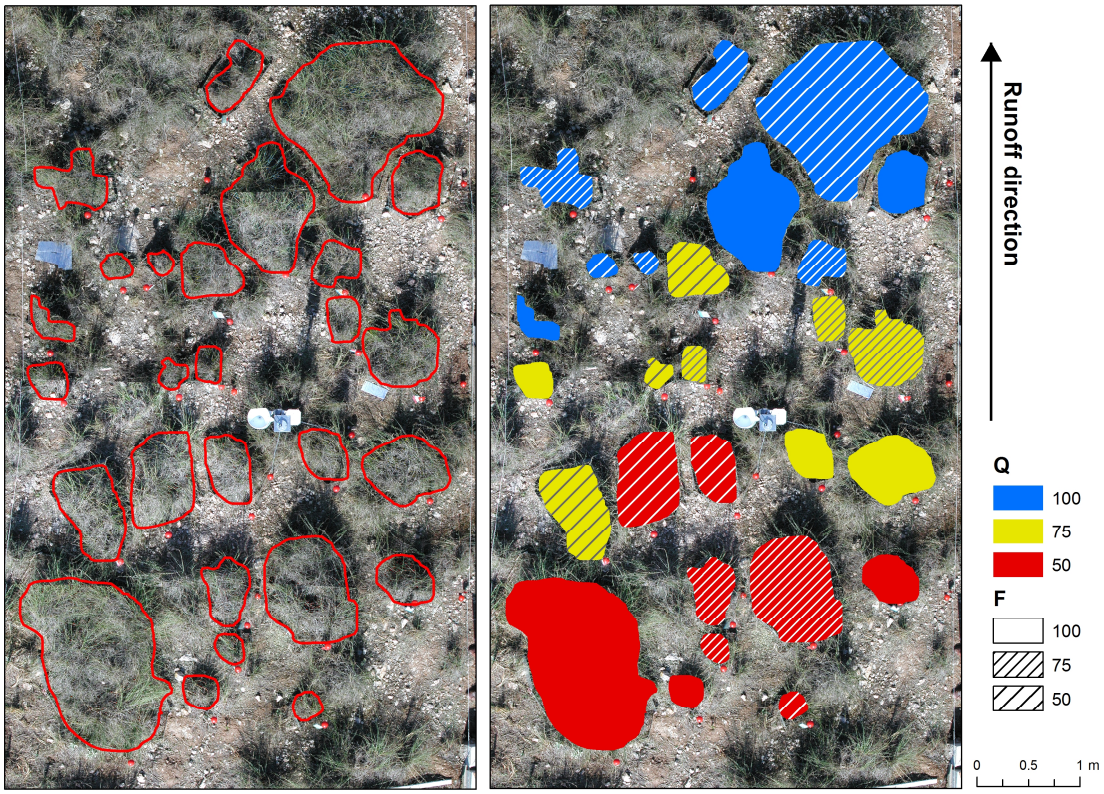

Figure S1 Photomosaic of the experimental plots photographed at a height of two meters.

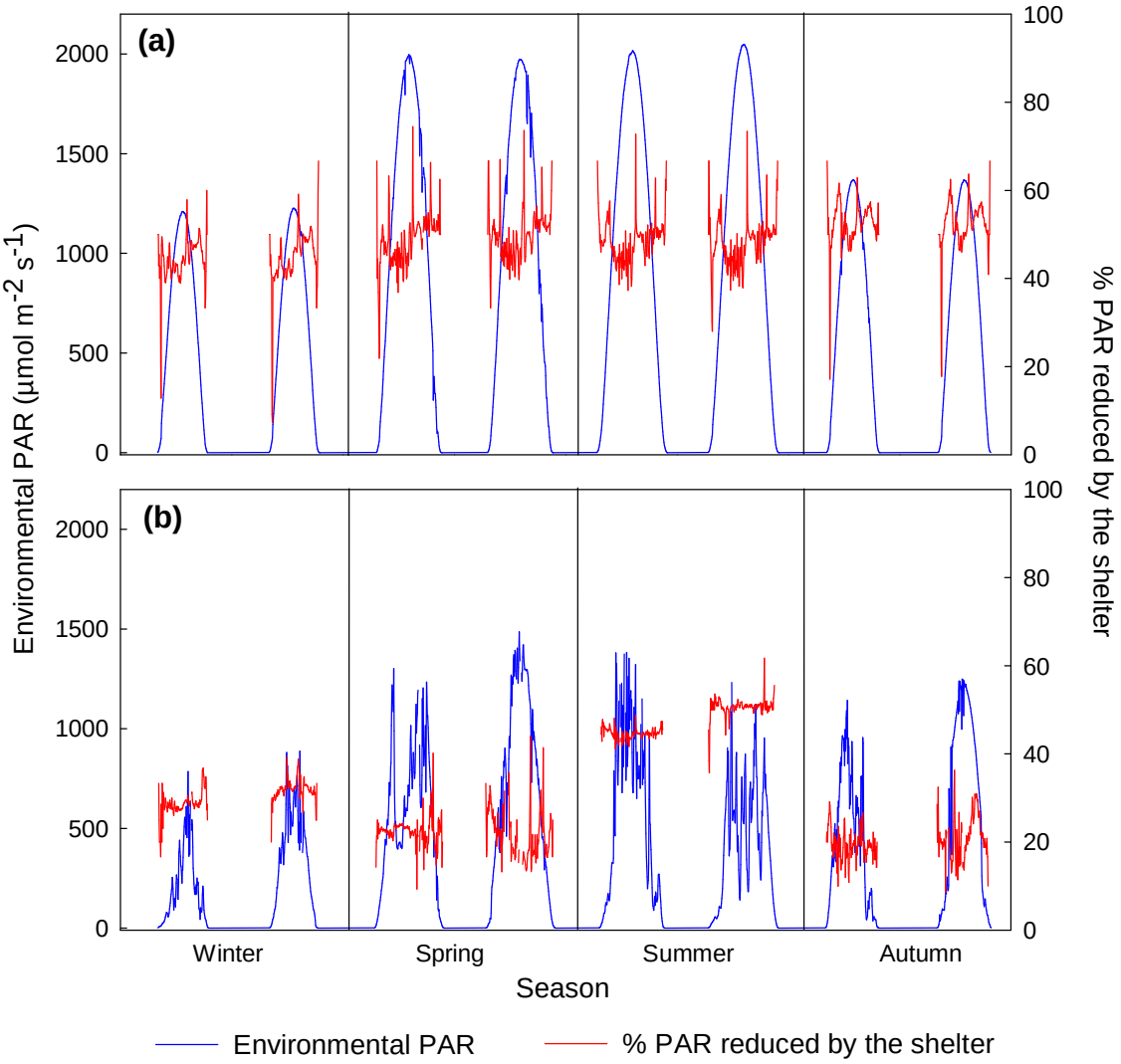

Figure S2 Examples of PAR on two sunny **(a)** and cloudy **(b)** days per month registered by a sensor outside the shelter (blue line), and the % of PAR reduced by the shelter according to a sensor located under it (red line).

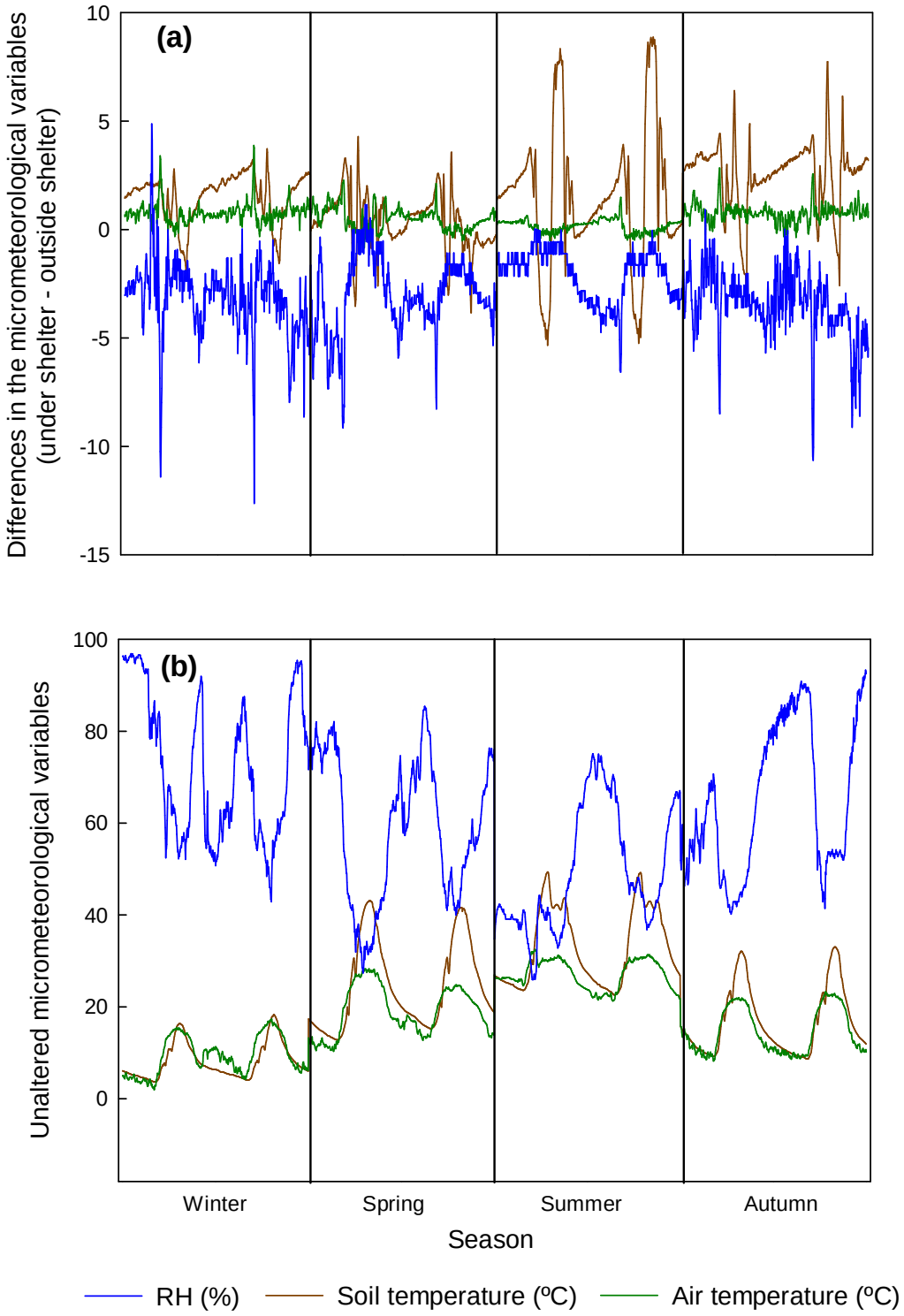

Figure S3 Examples of micrometeorological differences between inside and outside the shelter in two representative sunny days per season **(a)**, and natural evolution of the micrometeorological variables in the same days **(b)**.

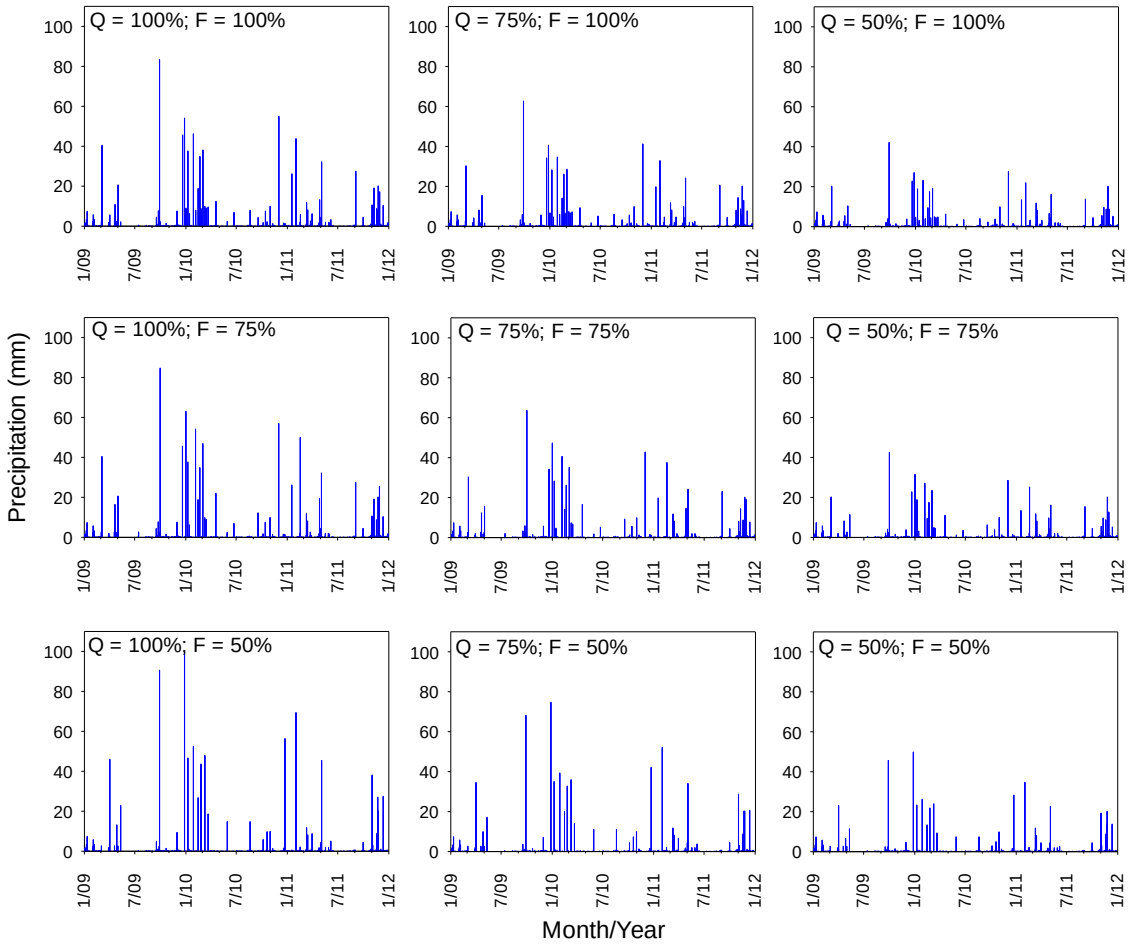

Figure S4 Watering schedule for each treatment according to the natural precipitation during the experiment. Q = quantity; F = frequency.

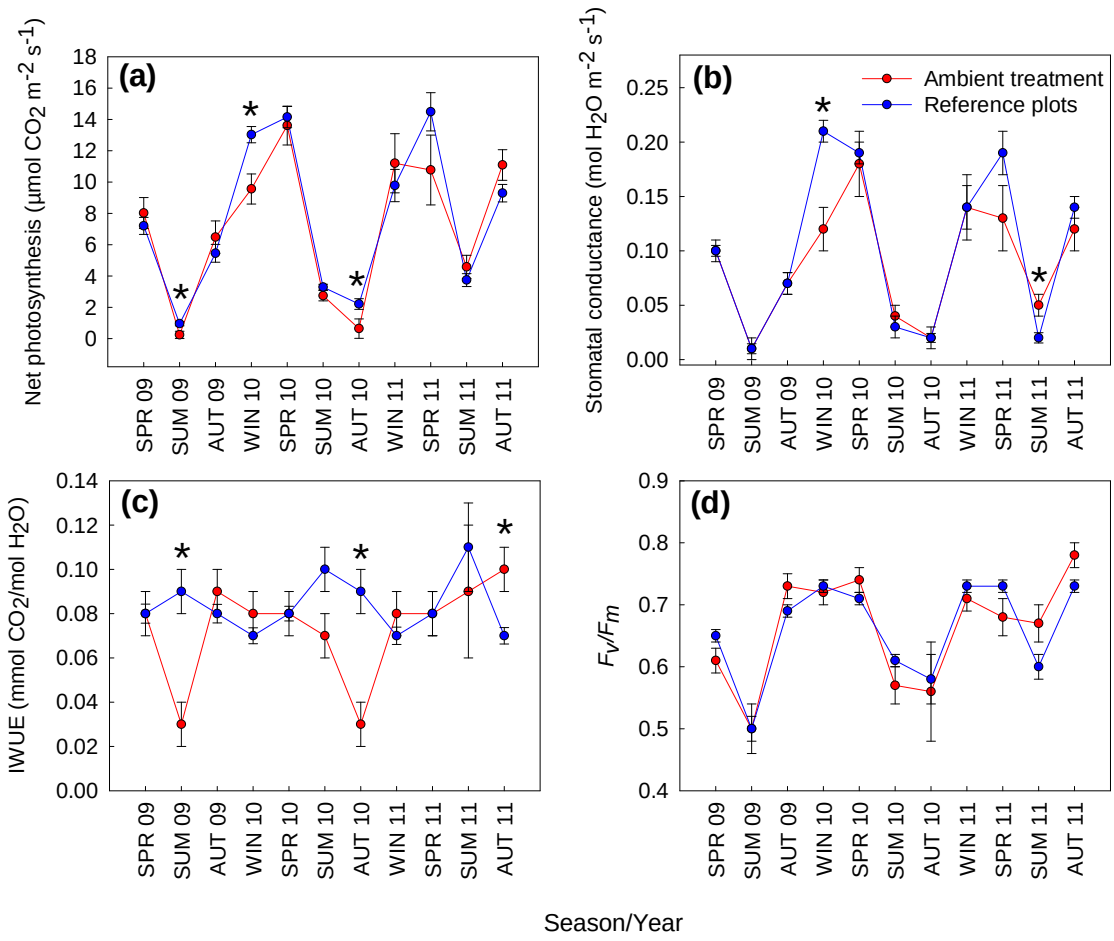

Figure S5 Fisher's least significant difference (LSD) post hoc seasonal-scale results for comparing the responses of ambient and reference plots in *A* **(a)**, g_s **(b)**, IWUE **(c)** and F_v/F_m **(d)** variables. Asterisks denote differences in means between ambient treatment and reference plots within each period at $p < 0.05$. Data are mean ± SE (n = 3).

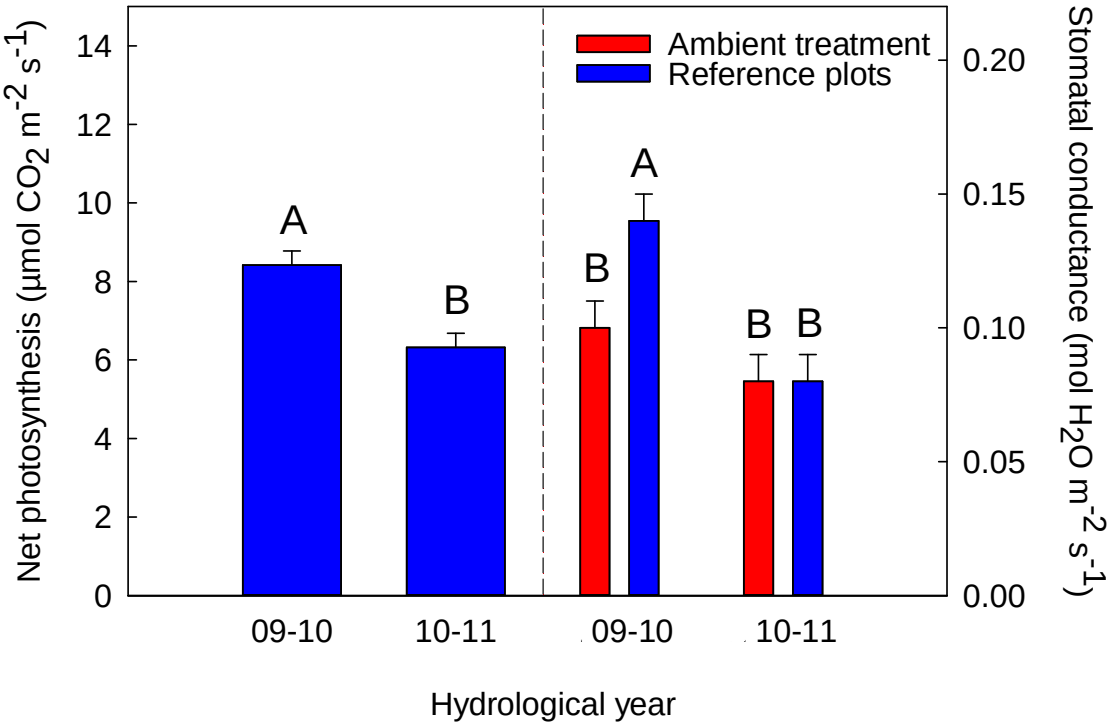

Figure S6 Fisher's least significant difference (LSD) post hoc annual-scale results for comparing the responses of ambient and reference plots in *A* and g_s grouping the months by hydrological years. Columns without letters in common denote differences in means between ambient treatment and reference plots at $p < 0.05$. Data are mean ± SE (n = 3).

Chapter II

Seeking photosynthetic response thresholds of *Macrochloa tenacissima* to water-pulses: implications for climate change

Ladrón de Guevara, M., Lázaro, R., Arnau-Rosalén, E. & Domingo, F. Seeking photosynthetic response thresholds of *Macrochloa tenacissima* to water-pulses: implications for climate change. *In preparation for publication.*

SEEKING PHOTOSYNTHETIC RESPONSE THRESHOLDS OF *MACROCHLOA TENACISSIMA* TO WATER-PULSES: IMPLICATIONS FOR CLIMATE CHANGE

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Abbreviations: *A*, net photosynthesis at leaf-scale; PAR, photosynthetically active radiation; LT, leaf temperature; Q, rainfall quantity; F, rainfall frequency; Q_P, accumulated quantity of irrigations within a period of 105 days; F_P, frequency of events within a period of 105 days; D_E, number of days between the last two events; Q_E, quantity of the last irrigation; P_S, plant size.

Abstract

In drylands, rainfall events play the major role in the ecosystem dynamics. Timing and quantity of individual rains are critical parameters for the operation of the system each biological season, and climate change studies forecast significant shifts in their patterns. Hence, we conducted a manipulative rainfall experiment that consisted of a total rainfall exclusion and irrigations on *Macrochloa tenacissima* tussocks using three levels of both water amount and event frequency (100%, 75% and 50%) regarding the natural rainfall pattern during the experimental period. *M. tenacissima* net photosynthesis at leaf-scale (*A*) was measured after each irrigation, and its response to rainfall features (disaggregated as irrigation attributes) was analyzed regarding three hierarchical temporal scales: (i) seasonality, (ii) antecedent precipitation conditions, represented by accumulated quantity of irrigations (Q_P) and frequency of events (F_p) during a period of time and (iii) current precipitation features, represented by number of days between the last two events (D_E) and quantity of the last irrigation (Q_E). The effect of the plant size (P_S) was also analyzed. The main implications of the response thresholds found regarding the forecasted shifts of rainfall patterns in the West Mediterranean Basin are: (i) an increase of days between events will not impair *M. tenacissima* response to water-pulses, provided it is accompanied by an increase of event size; (ii) however, a reduction of event frequency will have a negative impact on *A*; (iii) a decrease of the frequency of the small events will favor *M. tenacissima* performance; and (iv) *A* rates in early spring are greatly influenced by winter rainfall, so the evolution in quantity of precipitation in this last season will be the most critical for *M. tenacissima* growth.

Keywords: rain-out shelter; rainfall attributes; hierarchical timescales; seasonality; antecedent conditions; rainfall amount; rainfall frequency.

1. Introduction

The climate of Mediterranean areas is characterized by mild and wet winters and hot and dry summers. Precipitation patterns are key drivers of processes and ecosystem functioning of the arid and semiarid areas (hereafter drylands) present in regions under this climatic influence (Loik et al., 2004; Jongen et al., 2013). Although this climate is characterized by possessing a high variability of rainfall patterns (Luterbacher et al., 2006; Paredes et al., 2006), human activities and their indirect effects are moving terrestrial ecosystems outside of their historical range of variability (Seastedt et al., 2008). According to a Regional Climate Change Index (RCCI), the Mediterranean basin emerges as one of the primary hot spots of climate change (Giorgi, 2006), as even relatively minor modifications of the general circulation can lead to substantial changes of its climate (Giorgi & Lionello, 2008). Projected changes in the water cycle will lead to drier conditions in this area, caused by a decrease in the quantity and frequency of the precipitations, especially in the warm season (Christensen et al., 2007, 2013; Giorgi & Lionello, 2008), and an increase in the frequency and intensity of drought situations and extreme rain events (Stocker et al., 2013; Toreti et al., 2013). In fact, trends consistent with these forecasts have already been detected (e.g., de Luis et al., 2011; Sousa et al., 2011; van den Besselaar et al., 2012).

In drylands, ecosystem dynamics are essentially event-triggered (Puigde-fábregas, 1998). In fact, the precipitation pulses and their effects on these ecosystems can be rationalized in a hierarchical way as discrete events due to their large discontinuity, variability and unpredictability (Schwinning & Sala, 2004). That is, there is a relationship between the timing and magnitude of the event, the magnitude and extent of the ecological responses that it triggers and the time scale in which these responses take place. For this reason, the timing and quantity of individual rains are critical parameters for the operation of the system each biological season. For example, is especially important to vegetation performance the timing of the first relevant rains after the summer, the temporal separation and magnitude of rainfall pulses during the rainy season, and the extension of the wet season into spring (Noy-Meir, 1973; Beatley, 1974; Swarbreck et al., 2011). Biological activity dependent on the temporal evolution of these rain attributes has a repercussion in the ecosystem carbon and water fluxes. Despite the carbon fluxes from drylands ecosystems being relatively small, these

regions cover about 45% of Earth's land surface (Schimel, 2010) and contribute significantly to the global carbon budget.

However, few studies have focussed on the effects of the changes in dryland rain attributes on the dominant vegetation and its biogeochemical links (e.g., plant-atmosphere CO_2 and H_2O fluxes or carbon sequestration due to biomass production) within a conceptual framework of single-pulsed events or seasonal precipitation changes (e.g., Potts et al., 2006a; Miranda et al., 2011; Thomey et al., 2011). Historically, carbon-fixing and growth capacity of vegetation has been linked to the amount of annual rainfalls (Sala et al., 1988; Paruelo et al., 1999; Huxman et al., 2004). But rain manipulative experiments, which have been developed in the last decade as a popular alternative to the completion of long-term data sets to explore the response mechanisms of the ecosystems to the rainfall patterns (Yahdjian & Sala, 2002), also allow evaluating more rainfall attributes. For example, a synthesis of rain manipulative experiments highlights the negative effect of decreasing rainfall on the ecosystem net carbon uptake (Wu et al., 2011); but also a rapid response of grassland ecosystems to extreme rainfall regimes independent of annual precipitation amount (less frequency events but more water amount in each one) has been elucidated in a semiarid to mesic gradient (e.g., Knapp et al., 2002; Heisler-White et al., 2008, 2009, Fay et al., 2011). Net carbon uptake and growing responses vary along this rainfall gradient, evolving from positive in drylands to negative in mesic ecosystems (Knapp et al., 2008). Consequently, considering these studies, an antagonistic effect of amount decrease vs. frequency decrease is glimpsed in dryland ecosystems, being critical to determine the thresholds of response change of vegetation to the water-pulses configured by the interaction of these attributes.

Beatley (1974) formulated the concept of response thresholds to rainfall events as one of the ecology paradigms of arid and semiarid systems. But it was not until the twenty-first century when this idea gained importance in the study of these ecosystems (Schwinning et al., 2004). However, it is difficult to make generalizations about these thresholds because of the strong effects of the interaction between precipitation events, antecedent soil moisture, and specific plant functional types' responses, all of which are also affected by site characteristics and seasonality (Lázaro, 2004; Reynolds et al., 2004; Beier et al., 2012). In view of all this, the objective of this research was to determine the response thresholds of net photosynthesis at leaf-scale (A) to rainfall attributes (also considering as an attribute the characteristics of the antecedent rainfall),

and the changes of the responses with the interactions of these attributes, in a dominant plant of the Mediterranean drylands (*Macrochloa tenacissima* = *Stipa tenacissima*, alpha grass, esparto). We focused on the responses to individual events because the shallow roots of this species allow it to quickly change its physiological rates after single water-pulses (Pugnaire et al., 1996; Ramírez et al., 2007).

2. Materials and Methods

2.1. Site and species selection

The rainfall manipulation experiment was set up in the Balsa Blanca experimental site, a public land located in Cabo de Gata Natural Park, Spain (N36°56′26.0″, W2°01′58.8″) at 200 m a.s.l. This is a representative Mediterranean semiarid grassland dominated by *Macrochloa tenacissima*, the selected species of study. The mean annual temperature is around 18ºC, with mild winters, very sporadic frosts, and hot summers. A key characteristic of this dryland is that annual precipitation (ca. 220 mm $year^{-1}$) is greatly exceeded by evaporative demand, of approximately 1390 mm $year^{-1}$ (Rey et al., 2011). The warm season coincides with a period of prolonged drought (from May/June to September) with very infrequent, random and small rain events. *M. tenacissima* is a well-adapted species to the climate of this region, with several physiological and morphological traits that allow it to cope with tough conditions of water, temperature and radiation stress (see for more details Chapter I). Although this is a C_3 perennial herbaceous species, the intermittent and very variable rainfall throughout the year, which also causes fluctuating soil water content (SWC), provokes a temporal pattern of the green leaf area in this ecosystem, with a maximum peak in spring (Haase et al., 1999). The vegetation of the area is sparse, with about 63% of vascular plant cover (see some details of floristic composition in Chapter I), and where 90% of this vegetation is *M. tenacissima* (Oyonarte, pers. comm.). Open areas consist of bare soil, loose stones and biological soil crusts. Geomorphologically, the site is a series of alluvial fans (glacis) with gentle slopes (with a maximum of 8%) and substantially flat areas. Balsa Blanca soil is thin, with a variable depth (down to a maximum of ca. 30 cm, but on average 10 cm), and saturated in carbonates (Rey et al., 2011). It is classified as Calcaric Mollic Lithic Leptosol (WRB, 2006).

2.2. Experimental design

In February 2009, we started a rain manipulative experiment with the aim to test two factors and their interaction: quantity (Q) and frequency (F) of the rainfall, with three levels in each factor (100% regarding natural rain, and two reduction levels of 25% and 50%). These two factors were fully crossed, generating nine different treatments (as an example of used nomenclature, called Q100-F100, Q100-F75 … and Q50-F50), with three replicates for each one. Therefore, the total setup consisted of 27 plots of the target plant, *M. tenacissima*. Since in this species the size and age of the individuals influence their response to soil moisture (Ramírez et al., 2008a), the replicates of each treatment consisted of a small, medium and large individual with < 0.12 m^2, 0.12–0.25 m^2 and > 0.25 m^2 of projected canopy, respectively, to cover the entire range of variability of the responses for each treatment. Even though the terrain was almost completely flat, the plots were fenced with metal plates inserted into the soil (20 cm in height, standing out 10 cm) to prevent surface or subsurface water flow during the irrigations. The size of the plots was adapted to the surface covered per plant. In addition, the treatments that could generate more runoff due to the concentration of the rain events (Q100-F50) were placed on the bottom of the gentle slope (ca. 2–4º).

The treatments were carried out eliminating all natural rainfall and irrigating the plots between the first and the third day after each event. Rain exclusion was made with an automatic mobile shelter situated over the experimental plots only during rainfall events to avoid unwanted environmental alterations (see design specifications of the shelter infrastructure and the definition of rainfall event in Chapter I). Rainfall amount to irrigate after each event was calculated as the difference of two rain gauges, one situated in the middle of the experimental area, and another outside, in order to take into account the possible rain that fell on the plots before the shelter covered them. In each plot its area was used to convert the rained mm to ml to water. The frequency factor with the level 50% was performed with watering done once every two events and with the water amount of both events corresponding to the Q factor; and the frequency factor with the level 75% was performed with watering done for three consecutive events, no watering the fourth, and the saved water from the fourth event added to the next event.

2.3. Net photosynthetic activity (A) measurements

The measurements were performed between one to three days after the irrigation of the plots, the elapsed time where the maximal response to water-pulses takes place in this species (Pugnaire et al., 1996), and at the time with the highest *A* rates of the day, estimated according to previous full-day measurements conducted in different seasons. Leaf carbon exchange was determined with a portable infrared gas analyzer system (LI-6400; LI-COR, Lincoln, NE, USA) coupled to a chamber with LED light source (model 6400-02B, LI-COR). In each plant between four and six leaves, all facing north, were introduced into the chamber. The photosynthetically active area of each measurement was calculated by multiplying the width of the set of leaves introduced into the chamber by the length of the chamber. When the leaves were folded due to the water stress, the area was considered as a semicylinder, and the correction factor Π/2 was used. For more specifications about the gas exchange measurement protocol see Chapter I.

2.4. Statistical analyses

We analyzed the response of *A* to rain pulses, disaggregating the characteristics of the irrigation treatments into four attributes and considering three hierarchical temporal scales. The performance of plants in a Mediterranean climate is mainly determined by seasonal water stress (Manes et al., 1997; Balaguer et al., 2002; Serrano-Ortiz et al., 2015). Thereby, the first temporal hierarchy level was the seasonality (thereafter factor Season). Five different ways of grouping the data into seasons were checked: 1) in the four official seasons, 2) in six categories, using the inflection point of the *A* evolution during spring and autumn to divide these seasons into two periods with high and low activity, 3) in the four seasons, but including the periods of late spring and early autumn into the category of summer, 4) in three categories divided by periods of high (winter and early spring), intermediate (late spring and late autumn) and low (summer and early autumn) *A* rates, and 5) in two periods of high (winter, early spring, late autumn) and low (late spring, summer, early autumn) *A* rates. The second temporal hierarchy level was the antecedent rainfall characteristics, as antecedent environmental conditions may be more important than current conditions for plant, soil and ecosystem carbon exchange in semiarid systems (Ogle et al., 2015). Antecedent rainfall characteristics were integrated in the analyses through two irrigation attributes: accumulated water

quantity during a period prior to each measurement (Q_P), and frequency, i.e., number of rainfall events during the same period (F_P), testing the effects of different time periods in the statistical models by using Akaike's information criterion (AIC, Akaike 1973). The third temporal hierarchy level was the features of the most recent rain to the response, represented by another two irrigation attributes: water quantity of the last irrigation event (Q_E), and number of days between the last two events (D_E). These irrigation attributes were included in the models as covariates and the seasonality and plant size (P_S) effects were tested as fixed factors. The P_S categories used in the models were outlined in the experimental design section.

The analyses were implemented in InfoStat (ver. 2013p, http://www. infostat.com.ar.) using the R programming (ver. 3.1.0.) to build general linear mixed models (LMMs) with repeated measures. The analyses were running with the maximum likelihood (ML) procedure to choose the best combination of the way of grouping the data into seasons and Q_P and F_P covariates calculated with different periods of time prior to rainfall events. The minimum period of time tested was 15 days, increasing it in steps of 15 days until AIC values were not lower than in the previous period. Thus, the model with the lowest AIC value was selected. In Q_P, two analyses were implemented in each step to test the effect of the inclusion or exclusion of the event rain amount prior to the measurements. The models were running with all the possible interactions between the factors and covariates. The structures of variance/covariance of the models were chosen following also AIC values, but in this case running the analyses with the restricted maximum likelihood (REML) procedure. The absence of variance homogeneity and temporal autocorrelations were also checked by Pearson standardized residuals vs. fitted values plot, and autocorrelation function (ACF) plot, respectively. The absence of correlations between the covariates was tested with the variance inflation factor (VIF) parameter, and the normal distribution of the Pearson standardized residuals was verified by a QQ-plot. The effect of the inclusion or exclusion of the individuals (plots) as a random term on the temporal autocorrelation was also checked.

After choosing the best combination of seasonality mode and temporal length of antecessor rainfalls for the variable *A* (model with the lowest AIC), the most relevant interactions were selected. For this, the AICs of each interaction with the main effects were calculated, and a forward stepwise procedure was implemented, including an interaction in each step in an order of increasing individual AIC values. When the interactions were not significant (p-values > 0.05) they were excluded from the

procedure, but if the p-values were between 0.1 and 0.05, they were tested again when a new significant interaction was added in the model. When the final model with the lowest AIC and all the significant interactions was obtained, a final round with the excluded interactions was performed to check the effects of their inclusions on the AIC of the model, and their p-values with the final combination of interactions. If they did not improve the model and did not have a significant p-value, they were definitively excluded. Finally, the effects of the inclusions in the models of photosynthetically active radiation (PAR) and leaf temperature (LT) covariates (measured by the LI-6400) were also tested.

When the final model with the complete data pool was selected, the significant covariates were factorized by categorizing their continuous data in different ranges. This process allowed for determination of thresholds of the irrigation attributes with significantly different responses through the application of a post-hoc test (Fisher's least significant difference (LSD)). If the significant interactions of the full model were complex and crossing all the levels of the factors was not possible, at least one irrigation attribute was maintained in the factorial analysis as covariate. If seasonality was included in the significant interactions, intraseasonal analyses (with each season being analyzed separately) and interseasonal analyses (with similar data ranges of the irrigation attributes in several seasons) were performed. In the analysis of between seasonal effects, only the seasons that share similar ranges of data in F_P and D_E attributes were compared.

These factorial analyses were also performed using the LMM method with the same explained protocol. If the most complex significant interaction of the factorial analyses included a covariate, and LSD test could not be applied, its effects on the interaction of the different levels of the factors were evaluated graphically.

3. Results

3.1. Size and temporal distribution of the events

In our period of study, most of the events of the ambient treatment (Q100-F100) occurred in the range of 0.1 to 10 mm. 60% of the events had a smaller size than 10 mm, and 29% less than 5 mm (Fig. 1). The simulation of a more extreme rainfall regime (concentration of the events) together with a reduction of the annual amount had effects

on the size event distribution. The frequency decrease with the same rain quantity reduced the percentage of the smallest size events (under 5 mm) to 14%, and doubled the percentage of irrigations greater than 25 mm. The Q factor had an opposed effect on the rainfall event size, as in the treatment Q50-F100 the smallest irrigations (under 5 mm) supposed 57% of the total events. Thereby, the frequency of the smallest events was unaffected when the two factors are crossed in the most extreme level (Q50-F50). However, this treatment had an effect on the frequency of the medium-size events, as it decreased in favor of larger events. In the Level F100, 53% of the events had a temporal separation less than 10 days from the other events, but when F factor was reduced by 25% and 50%, this proportion decreased to 47% and 31%, respectively.

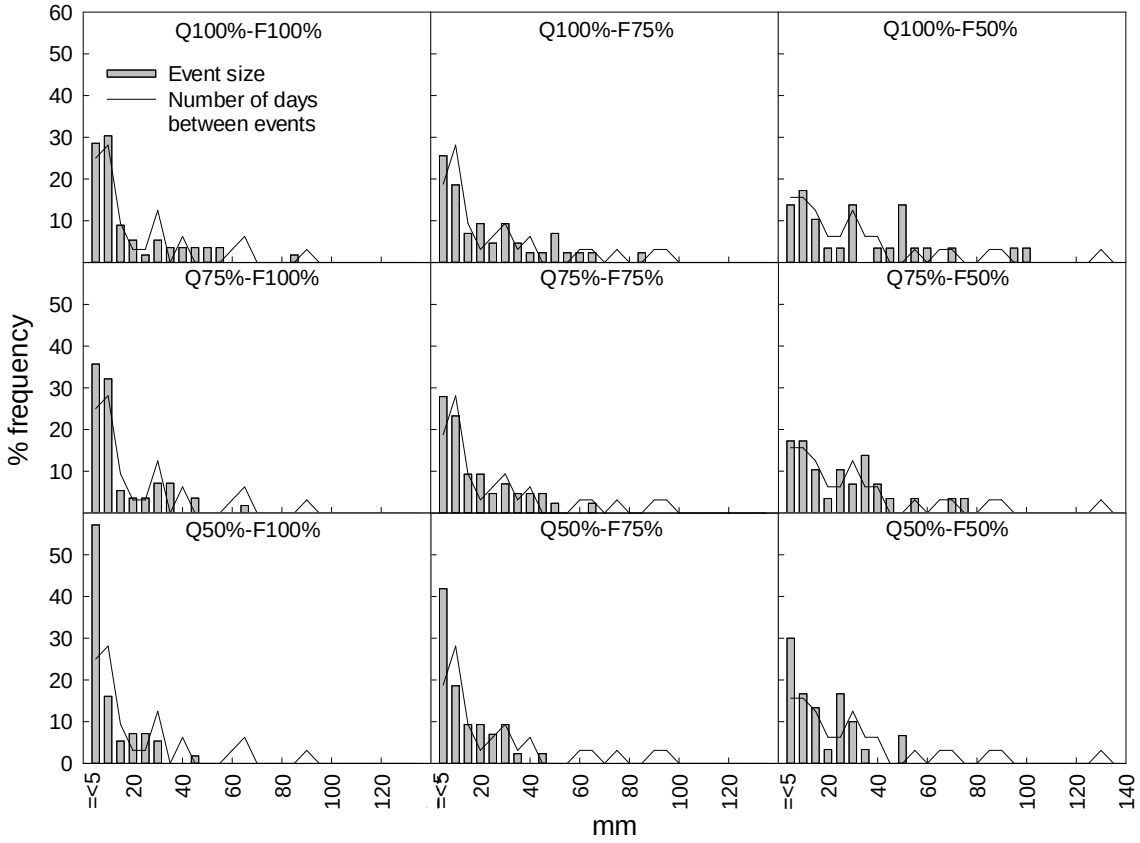

Figure 1 Effects of irrigation treatments on the frequency distribution of rainfall event size and number of days between events.

3.2. Final models and interactions of the irrigation attributors

The six seasonal categories and 105 days was the best combination between the factor season and periods of time of the antecessor rainfalls found. A better model was obtained when Q_P was calculated excluding the quantity of the prior event to the

measurements. Two significant complex interactions that involved all irrigation attributors as covariates were obtained in the final model with the complete data pool: Season × P_S × Q_P × D_E and Season × Q_P × F_P × Q_E × D_E (Table 1). Therefore, the intra and interseasonal effects of all irrigation attributes were analyzed categorizing the covariates and selecting the number and intervals of the categories using the AIC criteria in the ML models.

3.2.1. Interseasonal effects

The comparison of the seasons early spring, late spring and winter was possible in an interval of F_P between 4–10 and D_E between 4–9 and 30–68. When the irrigation attributes were factorized, the models did not run with more than one of them as a factor and they were maintained as covariates. Two five-term interactions were statistically significant, so they were chosen to graphically explore the relationship between the fixed terms of the model. Therefore, the three level timescales were present in the interactions. In the Season × Q_P × F_P × Q_E × D_E interaction, there was a positive relationship between *A* and Q_P in early spring, but being slightly positive or negative when more than 32 days elapsed between events (Fig. 2). When D_E did not exceed 8 days, the values of *A* increased sharply with Q_P until 150 mm, reaching an asymptote in this value (Fig. 2a). In late spring this relationship changed with the size of the last event, being positive when Q_E = 0 mm, negative when Q_E =1–5 mm, and slightly positive when Q_E = 10–25 mm (Fig. 2a, d, f). In winter, the positive relationships between *A* and Q_P were unclear, and in most of cases there was no slope in the regression line, or it was slightly negative (Fig. 2b, e, f, g). As in late spring, the greater negative slope occurred after small rain events (Fig. 2c). When Q_P was over 150 mm, *A* rates oscillated between 15–20 µmol CO_2 m^{-2} s^{-1} in early spring, even when the elapsed time between events surpassed one month; whereas this rate did not surpass the threshold of 15 µmol CO_2 m^{-2} s^{-1} in winter, being most of the values in the range of 5–10 µmol CO_2 m^{-2} s^{-1}. In late spring, most of the *A* values were between 1 and 5, regardless of the ranges of the irrigation attributes, indicating that the rates in this period were governed by the phenological cycle.

Table 1 Results of the MIXED model analysis of the irrigation attributes effects on net photosynthesis with all data pool. Season is composed of six categories, splitting spring and autumn in early and late periods; P_S = plant size; Q_E = event quantity; D_E = number of days between the last two events; Q_P = accumulated irrigation in a period of 105 days; F_P = frequency of events in a period of 105 days; LT = leaf temperature. Only interactions with p-value < 0.05 were included in the final model.

Irrigation attributes	DF	F-value	p-value
Season	5, 710	6.93	<0.001
P_S	2, 24	1.04	0.369
Q_P	1, 710	0.07	0.785
F_P	1, 710	3.77	0.053
Q_E	1, 710	0.64	0.423
D_E	1, 710	2.16	0.142
LT	1, 710	8.43	0.004
Season × Q_P	5, 710	2.96	0.012
Season × Q_E	5, 710	6.59	<0.001
Q_P × D_E	1, 710	11.6	0.001
F_P × D_E	1, 710	25.34	<0.001
Season × P_S × Q_P	12, 710	2.17	0.012
Season × Q_P × F_P	6, 710	5.41	<0.001
Season × Q_P × D_E	5, 710	2.54	0.027
Season × F_P × Q_E	6, 710	7.18	<0.001
Season × F_P × D_E	5, 710	9.00	<0.001
Season × Q_E × D_E	6, 710	13.23	<0.001
P_S × Q_P × D_E	2, 710	3.79	0.023
P_S × F_P × Q_E	2, 710	3.64	0.027
Q_P × F_P × D_E	1, 710	16.07	<0.001
Season × P_S × Q_P × F_P	12, 710	2.03	0.020
Season × Q_P × Q_E × D_E	6, 710	5.93	<0.001
Season × Q_P × F_P × D_E	5, 710	10.48	<0.001
Season × Q_P × F_P × Q_E × D_E	6, 710	2.78	0.011

In the Season × P_S × Q_P × F_P × D_E interaction, *A* rate decreased with the increase of the tussock size when Q_P > 150 mm, but in winter the values were similar between the size categories (Fig. 3). In early spring, the three plot sizes responded positively to Q_P when D_E was 4–8 days and F_P 4–6 events, although with less slope in small plots. However, when F_P increased to 7–8 events, only the activity of large plots had a positive relationship with Q_P, reaching higher *A* rates. In winter, only large plots had a clear positive response to Q_P, but in a different range of D_E and F_P (D_E = 26–30 days; F_P = 4–5 events), and without exceeding the *A* rate of the other sizes. However, in late spring only the medium plots presented a positive slope, also in the lower range of F_P (4–5 events), and with their *A* values in the same range as in the other plant sizes.

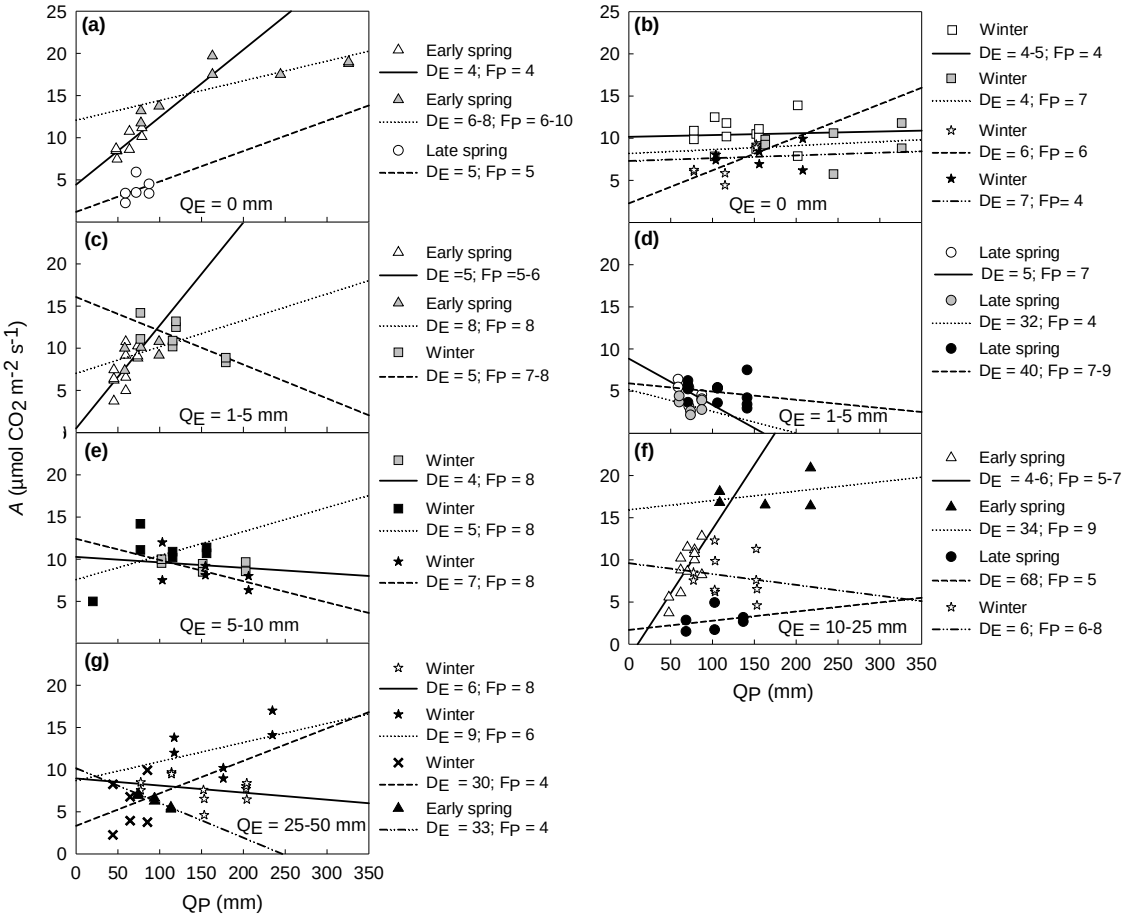

Figure 2 Comparison of the seasons early spring **(a**, **c**, **f**, **g)**, late spring **(a**, **d**, **f)** and winter **(b**, **c**, **e**, **f**, **g)** in the interaction of the irrigation attributes Season × Q_E × D_E × Q_P × F_P using combinations of F_P and D_E intervals present in the three seasons.

The intervals of F_P between 4–10 events and D_E between 11–19 and 21–38 days took place in early spring, late autumn and winter. As in the last interseasonal comparison, this analysis was performed with the irrigation attributes as covariates. Three significant interactions were found: Season × F_P × P_S × D_E, Season × Q_P × Q_E and P_S × Q_P × F_P. The first interaction showed that early spring was the season with a greater positive response to an increase of F_P, but this sensitivity decreased with the plant size (Fig. 4). In small and medium plants the regression slope between *A* and F_P was greater in the lower interval of D_E, whereas in the largest plants the slopes in the two intervals of D_E were quite similar. In late autumn and winter the regression slopes were slights or nonexistents. In these seasons, *A* values had similar ranges in the two intervals of D_E, except in the largest plants in early autumn, where the values were higher in the upper interval. In the interaction Season × Q_P × Q_E, in early spring *A* was more sensitive to Q_P shifts and in late autumn to Q_E (Fig. 5). In late autumn, when Q_E =

0 mm all measurements were below 5 µmol CO_2 m^{-2} s^{-1}, but when the plots received a recent water-pulse some values exceeded this threshold. In this season the greatest positive interaction with Q_P occurred when the event size was higher than 15 mm. In winter the greatest *A* values were obtained with a Q_P < 150 mm when Q_E < 45 mm. Under this last value the highest *A* rates were around Q_P = 150 mm, but when the accumulated irrigation increased, *A* decreased. In the interaction P_S × Q_P × F_P, only *A* responded to Q_P in the upper interval of F_P (Fig. 6). However, the differences between F_P intervals were likely influenced by the higher range of Q_P in the upper values of F_P. The increase of *A* with Q_P occurred from 175 mm, and only one measurement was done above this threshold in the lower interval of F_P. The magnitude of the response decreased with the size of the plants.

3.2.2. Intraseasonal effects

In early spring, the irrigation attributes of the second temporal scale that characterized the antecedent irrigation conditions, Q_P and F_P, interacted between them. The thresholds where F_P × Q_P caused a positive response in *A* was established in F_P = 6 and Q_P > 75 mm (Fig. 7a). When F_P = 4–5, an increment of Q_P did not cause a response in *A*, but when it rose to six, plants responded positively to Q_P > 75 mm. In the upper level of F_P, *A* rate was significantly higher than in the lower levels when Q_P > 75 mm, but F_P had any effect when Q_P < 75 mm. Therefore, there was a threshold of accumulated irrigations from which a linear positive relationship between *A* and F_P took place. In this season, these factors also interacted with their corresponding attributes in the event timescale (i.e., Q_P × Q_E and F_P × D_E). *A* was higher in the upper level of Q_P in the measurements without the irrigation of the previous event (Q_E = 0) and with an irrigation between 5–15 mm (Fig. 7b). When compared to the plots with Q_E = 0, no irrigation sizes caused a positive response in *A*, but some of them were negative when Q_P was in its upper threshold. As in the F_P × Q_P interaction, D_E had no effects on *A* when F_P was lower than 6 events (Fig. 7c). When F_P rose to six, an increase of D_E in the interval of 6–8 days caused a significant drop of *A*, but this response did not occur if the number of days exceeded this interval. This drop was likely influenced by the fact that these plots were not irrigated in the last event, whereas in the lower and upper interval of D_E, the plots had average Q_E of 8 and 11 mm, respectively. When F_P was higher than six, a significant increase of *A* took place at D_E = 11–24 days. In this case Q_P was partly

responsible for this high *A* value, as on average it was 238 mm, whereas in the other clusters, as a result of the $F_P \times D_E$ interaction, the averages of Q_P ranged from 48 to 166 mm. In conclusion, a lengthening of the days between events did not result in a loss of ability to perform photosynthesis in this species after great rain events, and within similar D_E values, *A* rate increased progressively with F_P, with its rate at the uppermost level being significantly higher than at the lower.

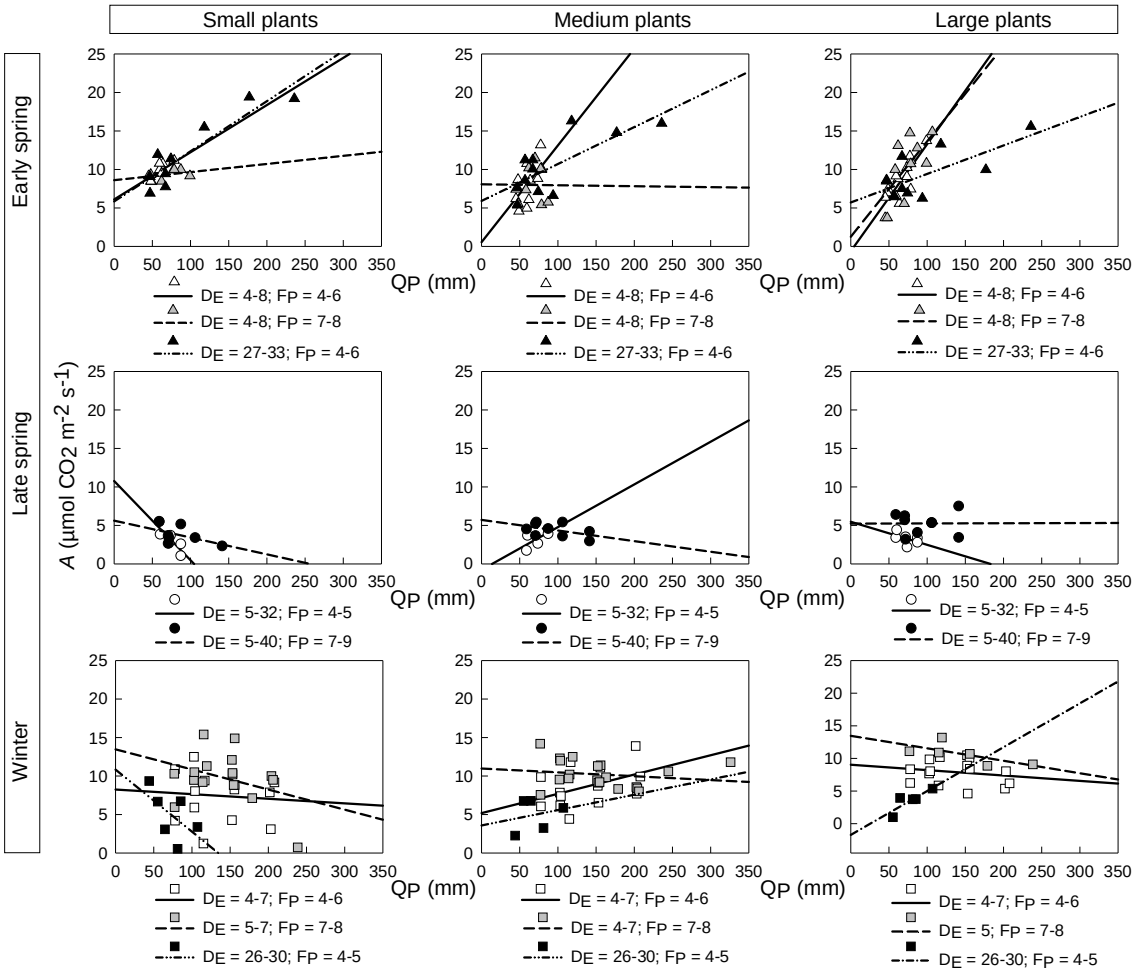

Figure 3 Comparison of the seasons early spring, late spring and winter in the interaction of the irrigation attributes Season × P_S × D_E × Q_P × F_P using combinations of F_P and D_E intervals present in the three seasons.

In late spring, the F_P attribute was significant in the statistical model, but without interactions (Fig. 7d). *A* in the upper level of this factor (7–9 events) was significantly higher than in the lower level (4–5 events). In the event timescale, Q_E interacted with P_S. Only small plants responded positively to an increment of Q_E when this attribute

was greater than 10 mm (Fig. 8a). But medium and large plants had greater rates in Q_E = 0 and in small events.

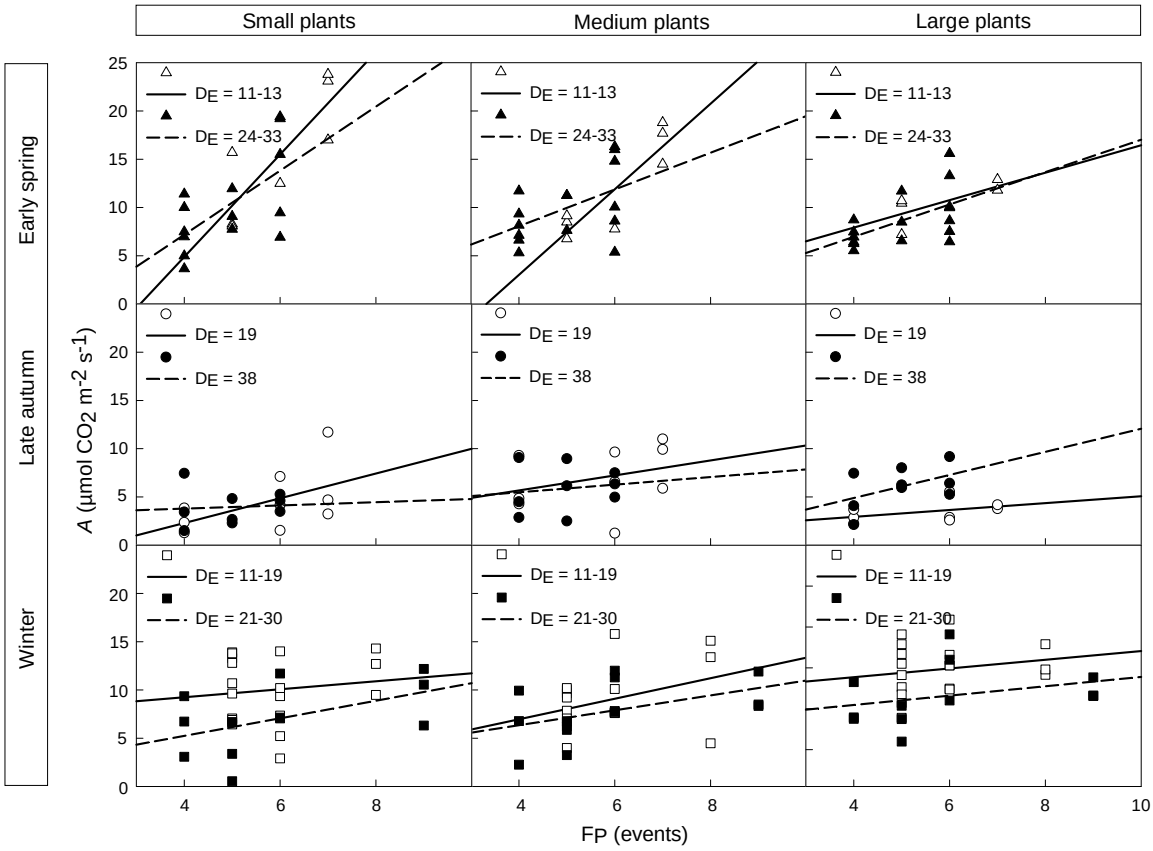

Figure 4 Comparison of the seasons early spring, late autumn and winter in the interaction of the irrigation attributes Season × P_S × D_E × F_P using combinations of F_P and D_E intervals present in the three seasons.

Q_E was significant in all seasons, but it had a relevant importance in summer, as this attribute was present in the three significant interactions found in the best model for this season (Q_P × Q_E × D_E, F_P × Q_E and Q_E × P_S). The response to Q_E was modulated by the irrigation attributes of the antecedent conditions. In Q_P, the established threshold of 75 mm in early spring dropped to 8 mm in summer. Due to the increase of elapsed days between events in this season, Q_P and Q_E also interacted with D_E. As in early spring, *A* was higher in the upper level of Q_P in the measurements without the irrigation of the previous event (Q_E = 0), but only when D_E did not exceed 64 days (Fig. 7e). A significant drop of *A* after small rain events (< 5 mm) took place, except in the interaction with the upper levels of D_E and Q_P. Unlike in early spring, a greater event size caused a significantly positive response in summer, but only in the interaction with the upper levels of D_E and Q_P. In this experimental design extending the drought period

also supposed a concentration of the rains in lower, but more intense events. This meant that the event sizes of the last irrigation when D_E was lower than 64 days ranged from 5 to 8 mm, whereas when it was higher they ranged from 5 to 30 mm. In conclusion, in summer events with greater size than 5 mm there was a positive response, but only when the last event was greater than 8mm. Regarding the significant $F_P \times Q_E$ interaction found in summer, the three event size categories had significantly greater *A* rate in the upper level of F_P (3–6 events) than in the lower (1–2 events). As in the previous interaction, the small irrigation size caused a negative response, in this case in the range of greater frequency of events; and this species also had a positive response to medium-large events in the upper level of F_P (Fig. 7f). Finally, Q_E also interacted with P_S in this season, where large plants had a significantly higher *A* rate than small plants when Q_E = 0, and than medium plants when Q_E > 5 mm (Fig. 7g). The three plant sizes suffered the drop of *A* when Q_E < 5 mm, but only small and large plant sizes responded positively when Q_E > 5 mm.

In early autumn, an irrigation attribute of the second timescale level, F_P, had two different interactions with the third timescale level ($F_P \times Q_E \times D_E$ and $F_P \times D_E \times P_S$). But Q_E was the primary attribute that determined the responses. An extreme event of 84 mm took place in one year (event with a D_E = 6 and Q_E = 40–84), following by a small one of only 2 mm (event with a D_E = 2). Those water inputs caused a relevant increase of *A*, but the previous event (D_E = 6 and Q_E = 3–7) did not promote *A* (Fig. 8b). In the next year another event with a maximum Q_E of 7.5 mm (D_E = 7) barely had an effect on photosynthesis. The effect of this extraordinary event was also reflected in the $F_P \times D_E \times P_S$ interaction (Fig. 8g, h, i). Before this event (when D_E = 6), the accumulated irrigation ranged from 2 to 7 mm, and the *A* was near the compensation point or even with negative values. However, in this D_E value there were also three *A* rates per plant size greater than 1 µmol CO_2 m^{-2} s^{-1} that correspond to the responses of the plants after the application of an irrigation with two events concentrated: the great event and a small event of 2 mm. The three large plants than received this treatment responded over 2 µmol CO_2 m^{-2} s^{-1}, and had the best relation with Q_P. In the measurements most recently performed after the extraordinary event (D_E = 2), large tussocks were the plants that showed a more clear relationship with Q_P, and therefore this was the size that took greater advantage of this situation to recover *A* rates after a prolonged drought.

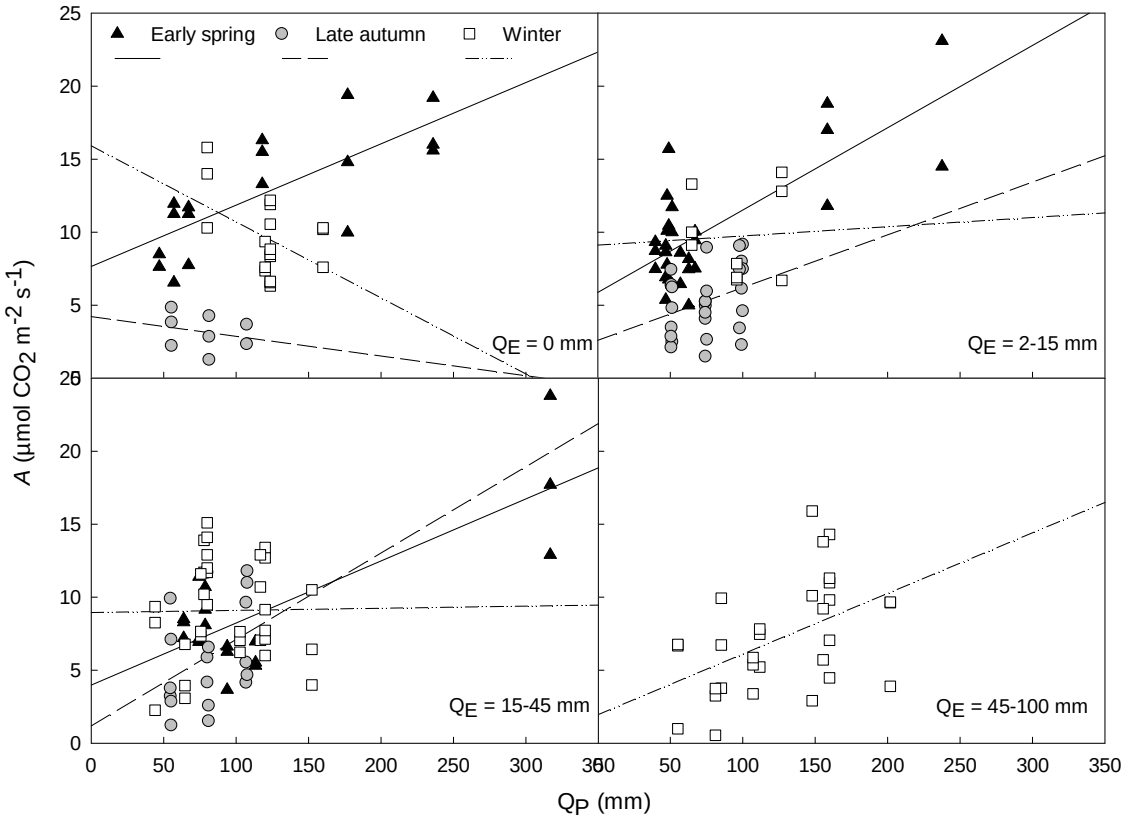

Figure 5 Comparison of the seasons early spring, late autumn and winter in the interaction of the irrigation attributes Season × Q_E × Q_P using combinations of F_P and D_E intervals present in the three seasons.

In late autumn, Q_P and F_P interacted with P_S and D_E, respectively. In addition, the two irrigation attributes of the third timescale, Q_E and D_E, interacted between them. The significant differences between plant sizes took place within the extreme Q_P levels (Fig. 7h). In the lower level (25–45 mm), the large-size plants had a significantly higher performance than the medium and small-size plants. In the upper level (80–110 mm), the small-size plants had lower performance than medium and large-size plants. Between Q_P levels, only significant differences were found in the small-size, with a greater *A* in a Q_P range of 45–44 mm than of 80–110 mm. In the F_P × D_E interaction, when D_E = 19 there was a positive relationship between *A* and F_P (Fig. 8c), but with a slight slope (1.28). When D_E increased to 38, the slope of the relationship decreased to 0.59 (Fig. 8d). However, as in this case the range of F_P values is lower, it is not possible to conclude clearly if with an increase of D_E *A* was less responsiveness to F_P. At event timescale, the major factor in Q_E × D_E interaction was the size of the events. *A* had a positive relationship with Q_E when D_E = 19 because the response threshold to irrigation sizes was located around 40 mm and in this D_E value there was an irrigation of 46 mm

irrigation. When the irrigations had lower entity (from 0 to 34 mm), the *A* rates were pretty similar. At D_E = 6, no relationship was found since the range of Q_E was between 0 and 20 mm (Fig. 8e, f). Therefore, the interaction of Q_E with D_E was due to a greater range of Q_E values in a particular D_E value rather than a change of *A* responsiveness to Q_E in a gradient of D_E.

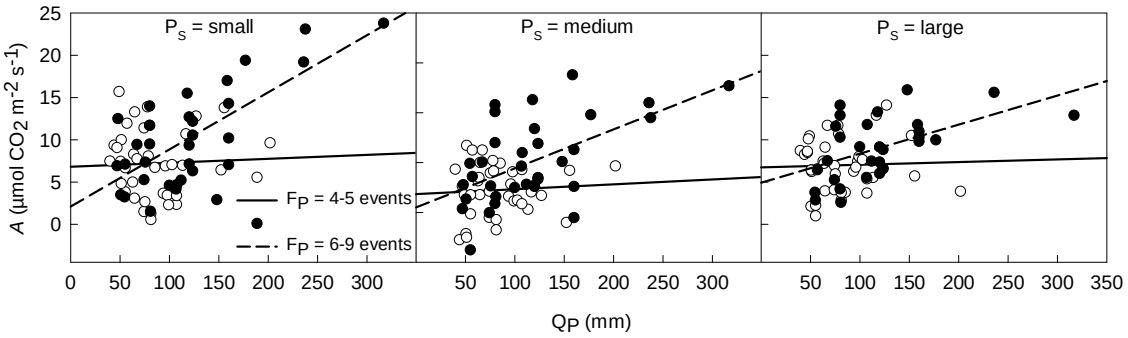

Figure 6 Representation of the interaction of the irrigation attributes $P_S \times Q_P \times F_P$ in the analysis of seasons early spring, late autumn and winter using combinations of F_P and D_E intervals present in the three seasons.

In winter, both Q_P and F_P interacted with D_E. In addition, F_P also interacted with P_S, and Q_E was significant without interacting with another attribute. In the $Q_P \times D_E$ interaction, the lowest rate was obtained when D_E = 5–6 days (Fig. 7i). Within D_E levels, only a differential response to Q_P was detected in the uppermost range (16–30 days). This D_E level responded positively to an increase of Q_P, causing the upper range (155–330 mm) a significant increase of *A*. This combination of D_E and Q_P also caused a significantly higher rate than those in all the levels of Q_P when D_E = 5–6, and in the lower and upper level of Q_P when D_E = 7–15. The non-linear response found in the irrigation attribute D_E in this season was caused by the influence of the entity of the last irrigation previous to the measurements. In the interval of Q_P =115–155 mm, the irrigations after elapsed days between events from 7 to 30 days were on average significantly greater than from 3 to 6 days (20–24 mm and 3 mm, respectively), so the deleterious effect of the prolongation of days without rain was counteracted by bigger and more concentrated irrigations. However, with a Q_P lower than 150 mm, the prolongation of days from 3–4 days to 5–6 days had a negative impact on *A* with smaller last events. The high *A* rate of the combination of Q_P = 155–330 mm and D_E = 16–30 was also influenced by the last event size (on average 53 mm), significantly higher than in the rest of the clusters formed by this interaction.

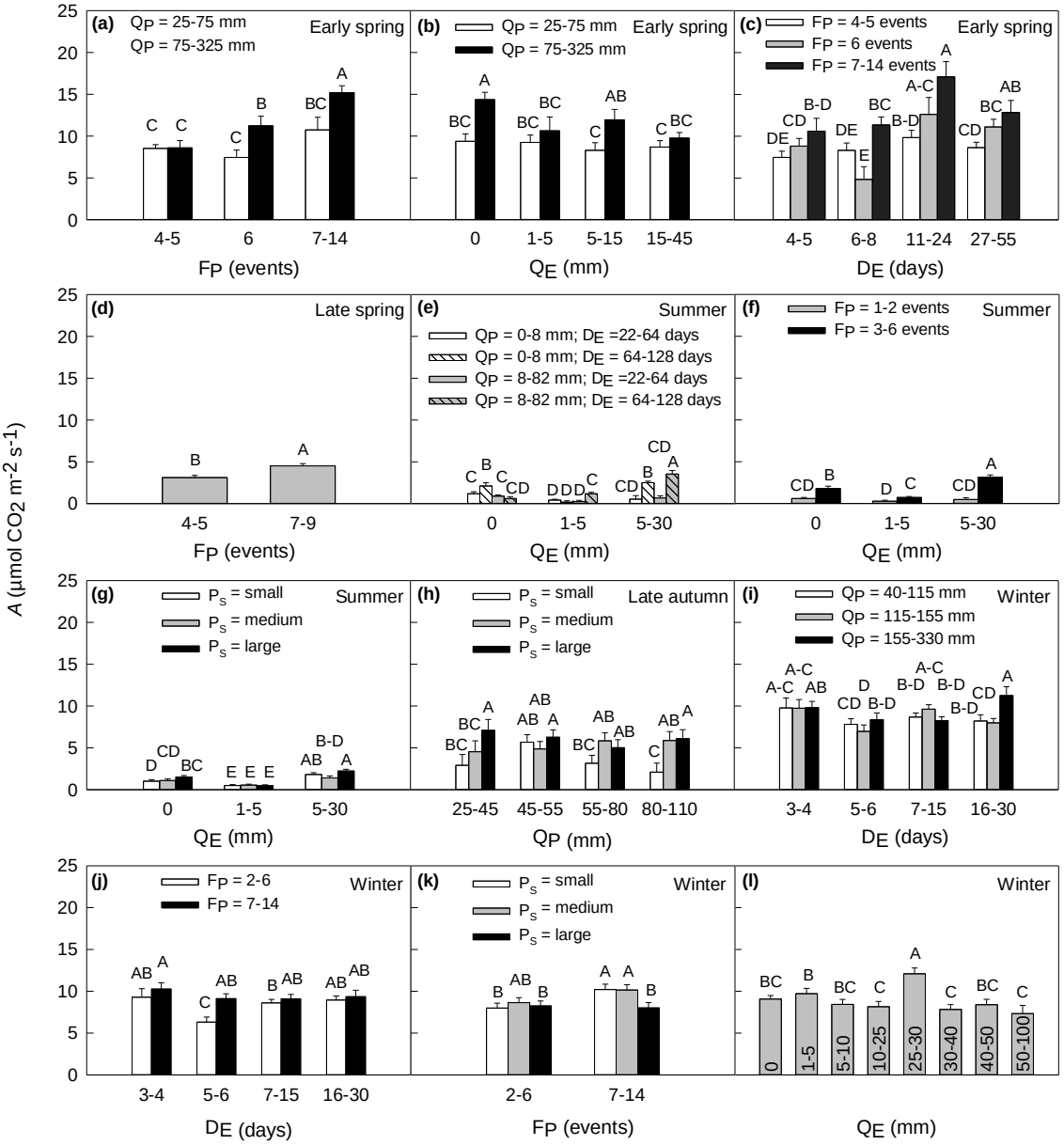

Figure 7 Fisher's least significant difference (LSD) post hoc results of the intraseasonal analyses for the interactions of the irrigation attribute Q_E **(a–e)** F_P **(f–j)** and Q_P **(k** and **l)**. Data are mean ± SE.

In the $F_P \times D_E$ interaction, only one category of D_E (5–6 days) suffered a significant decrease of *A* in the lower range of F_P (Fig. 7j). D_E = 3–4 days with the upper level of F_P had the greatest *A* rate, being significantly higher than the levels of D_E = 5–6 and 7–15 days in combination with the lower level of F_P. Regarding the $F_P \times P_S$ interaction (Fig. 7k), only small plants were sensitive to F_P, reducing their *A* when they were under a lower frequency regime of events. In the lower level of F_P there were no significant differences of *A* rates between plant sizes, whereas under the greater frequency regime larger plants had lower rates than the other size categories. Finally,

the best model was obtained when Q_E was factorized in eight categories. Comparing the seven categories of water-pulse sizes with non-irrigated plots in the last event, only one (25–30 mm) caused a significant increase of *A* (Fig. 7l). As this water size is located in the second percentile of the Q_E range, a non-linear response was found in this attribute.

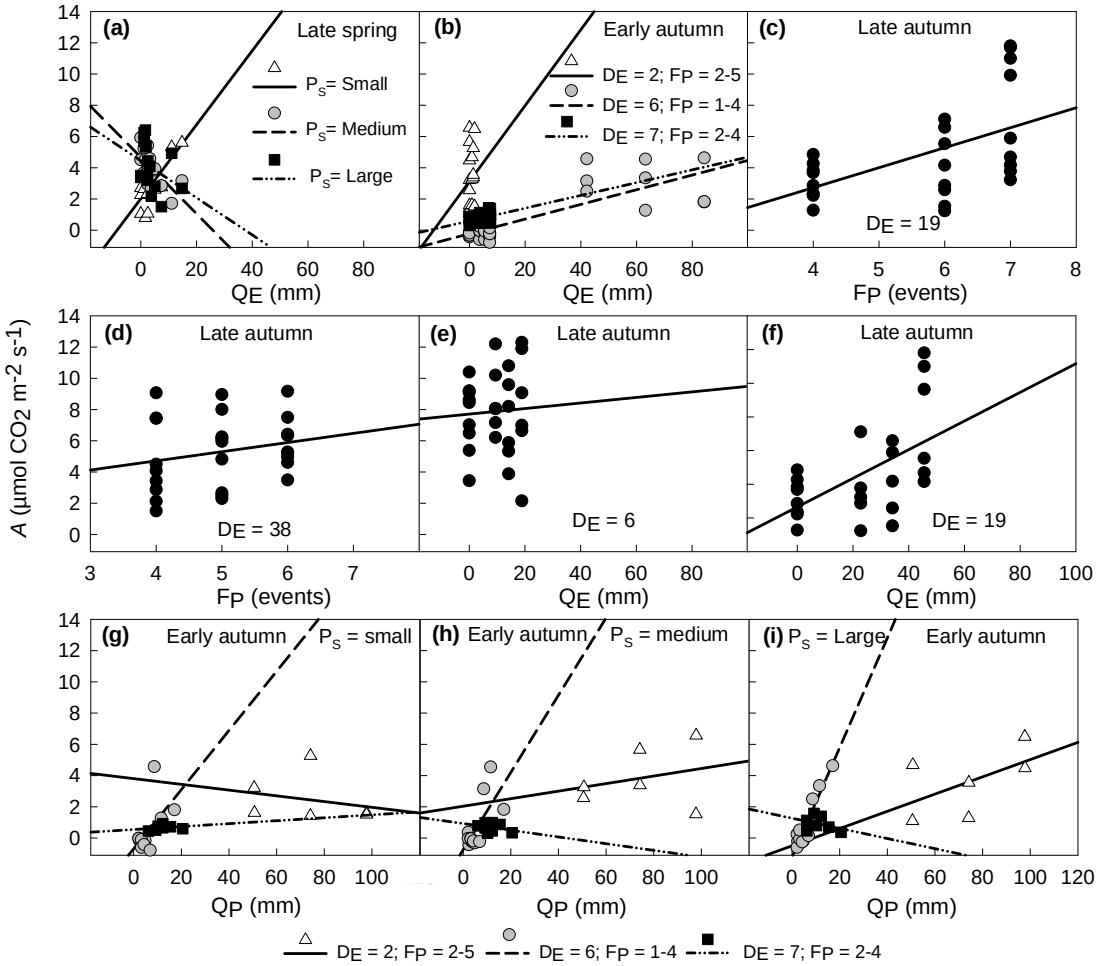

Figure 8 Representation of the significant interactions of the intraseasonal analyses when at least one irrigation attributes was defined as covariates in late spring **(a)**, early autumn **(b**, **g**, **h**, **i)** and late autumn **(c**, **d**, **e**, **f)**.

4. Discussion

The simulated rain patterns (decreasing rainfall amount and frequency of the events, causing an increasing of rainfall intensities and intervals between events) represent the most important features of climate change to be a precursor of aridity in climatic transition regions (Lavee et al., 1998), and the ability of semiarid Mediterranean plants to cope with a changing environment could be critical in the magnitude of future desertification processes. When the complete data pool of three rain manipulation years

was analyzed, all irrigation attributes interacted with the factor Season. Thereby, under this climate gas exchange must be analyzed in different seasons to better understand the relationship between carbon flux and water availability (Domingo et al., 2011).

M. tenacissima is a drought tolerance species, ability attributed to its capacity for seasonal acclimation and adjustment of its physiology (e.g., Domingo et al., 1991; Haase et al., 1999; Ramírez et al., 2008b). What is more, this species is able to endure a severe dehydration of its leaves losing up to 50% of leaf chlorophyll and turning green after the first autumn rains (Haase et al., 1999; Balaguer et al., 2002), so it fits the definition of a desiccation-tolerant plant with a poikilochlorophylly strategy. As in other Mediterranean species (e.g., Flexas et al., 2001; Gulías et al., 2009; Prieto et al., 2009), water availability is the most relevant abiotic factor in *M. tenacissima* activity, and therefore seasonality, acting both through water availability and plant phenology, played a main role in its relations with irrigation attributes. Perennial Mediterranean plants have in general two peaks of *A* in spring and autumn and minimum rates in summer (e.g., Ogaya & Peñuelas, 2003; Prieto et al., 2009; Gimeno et al., 2012). However, in this species *A* was still quite low in early autumn (Fig. 9) because its photosynthetic apparatus could not operate at full capacity due to the loss of an important % of leaf chlorophyll during late spring and summer. But the minimization of photo-oxidative damages and not having to maintain an intact photosynthetic system through long inactive periods of desiccation probably outweighs the disadvantage of slower *A* recovery and the energy cost of reconstructing internal chloroplast structures (Toldi et al., 2009). In this species, the recovery of *A* after the drought season was progressive from autumn to spring, and not bi-modular (two peaks in autumn and spring with a depression in winter) as in other Mediterranean regions (Flexas et al., 2014) due to the milder winters in this area of study. Although *M. tenacissima* has the capacity to respond quickly to water-pulses (Pugnaire et al., 1996), its seasonal phenology and specific weather conditions also influenced the carbon fixation.

Our results allow appreciating different factors that modulate the responses to water inputs in a temporal hierarchy. The first and main temporal hierarchy level that modulates lower levels was the seasonality, and thus the responses to antecedent rainfalls and single water inputs were different in the interseasonal analyses. For example, this species responded more clearly to Q_P in early spring than in other seasons (Fig. 2), this attribute being more important than Q_E in this season. This result agrees with the study of Llorens et al. (2004), where a positive relationship between *A* rates

and accumulated rainfall over the greatest growth period was found in a northern to southern gradient. Therefore, in *M. tenacissima* the winter precipitations are key to its carbon fixing capacity, and hence growth in early spring. In late spring a sharp down-regulation of *A* took place (Figs. 2, 3) and there was no response neither to Q_P nor Q_E when discrimination by size was not implemented. This phenomenon was due to the beginning of a process of chlorophyll loss (Balaguer et al., 2002) and a decrease of stomatal conductance due to a progressive increase of temperature and water stress and a folding of the leaves (Pugnaire et al., 1996; Fig. 9, own data). Indeed, *A* of *M. tenacissima* is more sensitive to stomatal regulation than another perennial species of the region (Domingo et al., 2002). In winter, the lower response to Q_P was likely associated with low temperature limitations (on average 11.5ºC), as stomatal conductance and F_v/F_m reach relatively high values in this season (Fig. 9; Pugnaire & Haase, 1996; Ramírez et al., 2009; Chapter I of this thesis), and most Mediterranean plants show an optimum temperature for photosynthesis in a range of 15–30ºC (Flexas et al., 2014). Beatley (1974) notes that to trigger the biological activity after the summer drought, an event greater than 15 mm is necessary, and it has to exceed 25 mm to be effective in the vast majority of plants. A similar response threshold was found in this study (Fig. 8b, g, h, i), but as autumn progressed events below these thresholds were able to cause a response of *A* (Fig. 5).

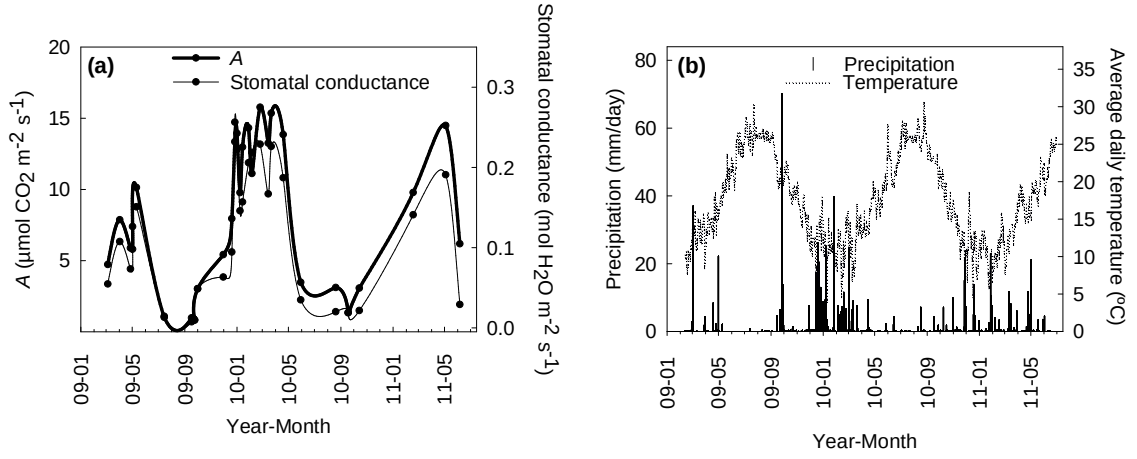

Figure 9 Average gas exchange activity of reference plants under natural conditions (n = 10) during the experimental period (**a**) and meteorology (temperature and precipitation) during the same period of time (**b**).

Recent studies have highlighted the critical role of antecedent conditions (considered in the second temporal level) in the responses of vegetation and soil

respiration in several intra-annual timescales (e.g., Cable et al., 2013; Barron-Gafford et al., 2014; Ryan et al., 2015), but its importance in photosynthetic activity has been less evaluated. The best period found for analyzing antecedent conditions (105 days) is similar to that used in the study of Llorens et al. (2004). In our research, antecedent characteristics of the rainfall (represented by the attributes Q_P and F_P), were modulated by seasonality, as for example in spring there is a strong phenological component (e.g., more resources for sexual reproduction, start of chlorophyll loss) and seasonal temperatures can also affect *A* responses to water-pulses. This led, as explained above, to Q_P having different effects on the response to rain events over the seasons. But at the same time, this timescale modulated the response of *A* to Q_E, especially in early spring and summer, increasing the responsiveness in the upper range of Q_P and F_P attributes (Fig. 7).

Regarding the third temporal level, previous studies have pointed out that a rain regime with frequency reduction and event size increase promote a greater productivity and photosynthetic activity in drylands (Heisler-White et al., 2008, 2009; Thomey et al., 2011). In general, morphological patterns of dryland species allow to them make use of rainfall pulses that become more intense and less frequent (Ramírez et al., 2012). In our study, a discrimination of responses between these two rainfall attributes (frequency and size) was performed, and it was possible to show that they involve opposite responses. In this experimental design an increase of D_E implied a greater size of the last event prior to the measurements. Therefore, the effects caused by D_E corroborated the above assumption (Fig. 7c, e, i, j). But on the other hand, the effect of reducing F_P did not have a positive effect on *A*, but quite the contrary, even when a lower F_P also supposed a greater intensity of the events (Figs. 2a, g; 7a, c, d, f, j). When the data pool was analyzed without separating these factors at an annual scale, a decrease of *A* activity was observed when rain frequency was reduced and event size increased (Chapter I), indicating that the effects of frequency shifts prevails over size shifts of the last event in these temporal scales.

In dryland regions, small precipitation events are frequent (Sala & Lauenroth, 1982; Lázaro et al., 2001; Reynolds et al., 2004; Magliano et al., 2015), and the major consequence of this rainfall regime characteristic is that the individual small events remain in the upper soil layer, which is more vulnerable to evaporation (Loik et al., 2004; Schwinning & Sala, 2004; Heisler-White et al., 2008). In summer, this species is chronically photo-inhibited due to the combination of high irradiance, water deficit and

high temperatures (Valladares & Pugnaire, 1999; Balaguer et al., 2002; Armas & Pugnaire, 2005). But in drought conditions ($D_E > 30$; $Q_P < 150$), Q_E greater than 5 mm triggered *A* (Fig. 7e, g, f) since herbaceous plants can use primarily shallow soil water from recent rainfalls (Breshears & Barnes, 1999; Schwinning et al., 2002; Ogle & Reynolds, 2004), which also occurs in *M. tenacissima* (Haase et al., 1995). The shallow root system of *M. tenacissima* allows it to acquire water in this soil layer, but the results of this research showed that in most cases, especially in summer, small events caused a decrease of *A*, likely because they promote a greater activation of respiration or photorespiration than photosynthesis. But on the other hand, excessive precipitation reduces the partial pressure of oxygen around the roots of plants, which usually causes a decrease of their hydraulic conductivity, thereby reducing water uptake (Davies, 2007). As a result of this, leaves can also experience temporary water shortage when the soil water content is high (Jackson et al., 1996; Vartapetian & Jackson, 1997). This could explain why the most effective event size was greater than 40 mm in late autumn, between 25–30 mm in winter, and between 5–15 mm in early spring; i.e., as soil moisture increases after the drought season, the size of the most effective event for photosynthesis decreases (Fig. 7b, l, 8f).

We also found different responses depending on the plant size in all seasons. In late spring, when the evaporative process gradually increases and the physiology of this species begins a process of adaptation to the coming drought period, the response to event size did not interact with Q_P, but with P_S (Fig. 8a). Only small plants responded positively to the increase of water-pulse, probably because bigger sizes relocated photosynthetic resources for the formation of reproductive tissues, sometimes even causing death of leaves (Sánchez, 1995). For example, 2010 was a masting year and the small, medium and large plants of our experiment produced 0, 6 and 20 spikes, respectively. However, from summer to late autumn (the period of higher water, light and temperature stress), the greatest tussock size was able to take advantage of its more developed root system and its canopy with a more effective self-shading photoprotection (Valladares & Pugnaire, 1999; Ramírez et al., 2008b) to keep a greater activity in situations of non-irrigation than small plants, or to respond with a higher *A* rate to different magnitudes of Q_E and Q_P (Figs. 7c, k; 8g, h, i). Conversely, in winter and early spring small and medium plants with high F_P had greater *A* than large plants, this difference being more relevant when D_E was lower than 15 days in early spring (Figs. 4; 6; 7j). In agreement with these results, Ramírez et al. (2008b) also found that

the largest *M. tenacissima* tussocks of their samples had significantly greater and lower *A* rates in summer and winter, respectively. Armas & Pugnaire (2005) found higher gas exchange activity in juvenile tussocks in early summer, but due to unusual rainfalls the water stress was lower than the corresponding in this season. Hence, in addition to its greater water use efficiency (Ramírez et al., 2008a, b), larger tussocks develop a below and above architecture more effective in carbon fixing during harsh environmental situations (i.e., a greater root system and a photoprotective canopy architecture), whilst small and medium size are more vigorous when the conditions are more favorable for photosynthesis. Higher carbon fixation capacity of large tussocks has been found in other species under elevated irradiation environment (Caldwell et al., 1983), so this response could be applicable to most species of dryland regions with this architecture.

A responsiveness to event size and frequency could be greatly influenced by the specific features of the regional site (Beier et al., 2012), as for example Thomey et al. (2011) did not find a differential response to these factors in a dryland ecosystem with dry cool winters and springs and most of the water inputs in summer; and Wertin et al. (2015) detected much lower effects than expected in a semiarid ecosystem with warm summers and cold winters, both of them with water inputs. However, in a similar ecosystem of Thomey's study, several annual species had different species-specific responses to two treatments of rain size and temporal distribution in relation to their biomass (Gutierrez et al., 1988), one of them increasing its biomass with the concentration of precipitations, and another one increasing it with more frequent smaller size events. Hence, the response thresholds, in addition to being species-specific, cannot be generalized out of the climatic region.

5. Final remarks: implications of the found responses facing climate change

Obtaining clear response thresholds to rainfall attributes was complicated due to the common non-linear temporal responses of the processes in this ecosystem type (Potts et al., 2006b) and the physiological variability of this species depending on the size and age of individuals (Armas & Pugnaire, 2005; Ramírez et al., 2008a, b). However, the following significant thresholds, greatly modulated by seasonality, have been found: in winter, under a specific Q_P (115–155 mm) an increase of dry days from 3–4 to only 5–6

had a negative impact on *A* when the last event was small. This threshold of 4 to 5 days delimits the deleterious effect of increasing days between events without increasing their size. However, when a greater amplification of the dry period regime was accompanied by an increase in event sizes, nonnegative effects were found in photosynthetic rates.

In summer, the increase of event size promoted significantly higher *A* rates with a very low threshold of accumulated rains (Q_P must be greater than 8 mm), and even elapses between events of 128 days have not had negative repercussions on the recovery of *A* since *M. tenacissima* is better suited to cope with water shortage than another species with similar morphological features (Pugnaire and Haase, 1996). However, small events caused a greater activation of respiration or photorespiration than photosynthesis, especially in summer. The fact that it is in this season when this effect is more acute could mean an increase in photorespiration, as the enzyme rubisco has greater affinity for O_2 at high temperatures (Brooks & Farquhar, 1985). This finding implies that the forecasted reduction of number of events will not reduce the responsiveness of *M. tenacissima* to water-pulses if it is accompanied by a significant increase of the event size, and even a decrease of the frequency of < 5 mm event size could enhance the net carbon assimilation in *M. tenacissima*.

However, the forecasted reduction of total rainfall amounts also will cause a reduction of the *A* rates, and will have more repercussions on early spring activity, where the rates are the highest and a deleterious effect on them was detected when Q_P < 75 mm. This implies that the evolution of winter rain quantities will be especially important in the growth and carbon fixation capacity of this species. The first great event after the summer also determines the start of the growth period. This study showed that an event of 7.5 mm did not promote *A* rates, but an event of 40 mm could double their values. However, a more accurate threshold of the event size that triggers the progressive recovery of *A* along this season was not possible to find. These responses are consistent with the determination of growth periods at ecosystem scale (Serrano-Ortiz et al., 2014), where only continuous growth period was detected in winter, with shorter discontinuous intervals in early spring and late autumn.

Finally, the results of this study showed a physiological memory of this species regarding frequency and amount of rainfall events in a period of time of at least 105 days. For example, in early spring *A* increased progressively with F_P when Q_P > 75 mm. Previous analyses of seasonal and annual *A* averages have demonstrated that a reduction

of event frequency decreases *A* rates, overcoming the positive effect of an intensification of water-pulses. However, *A* only evaluates momentary physiological ability to fix carbon, and a better assessment of the effect of rain frequency over time could be done by monitoring accumulative measurements of carbon fixation, such as growth capacity and biomass evolution.

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Chapter III

Easy-to-make portable chamber for *in situ* CO_2 exchange measurements on biological soil crusts

Ladrón de Guevara, M., Lázaro, R., Quero, J.L., Chamizo, S. & Domingo, F. (2015). Easy-to-make portable chamber for *in situ* CO_2 exchange measurements on biological soil crusts. *Photosynthetica*, *53*, 72-84.

EASY-TO-MAKE PORTABLE CHAMBER FOR *IN SITU* CO_2 EXCHANGE MEASUREMENTS ON BIOLOGICAL SOIL CRUSTS

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Abbreviations: BSC, Biological soil crust; IRGA, Infrared gas analyzer; RH, air relative humidity.

Abstract

Commercial chambers for *in vivo* gas exchange are usually designed to measure on vascular plants, but not on cryptogams and other organisms forming biological soil crusts (BSCs). We have therefore designed two versions of a chamber with different volumes for determining CO_2 exchange with a portable photosynthesis system, for three main purposes: (i) to measure *in situ* CO_2 exchange on soils covered by BSCs with minimal physical and microenvironmental disturbance; (ii) to acquire CO_2 exchange measurements comparable with the most widely employed systems and methodologies; and (iii) to monitor CO_2 exchange over time. Different configurations were tested in the two versions of the chamber and fluxes were compared to those measured by four reference commercial chambers: three attached to two respirometers, and a conifer chamber attached to a portable photosynthesis system. Most comparisons were done on biologically crusted soil samples. When using devices in a closed system, fluxes were higher and the relationships with the reference chambers were weaker. Nevertheless, high correlations between our chamber operating in open system and measurements of commercial respiration and photosynthetic chambers were found in all cases ($R^2 > 0.9$), indicating the suitability of the chamber designed for *in situ* measurements of CO_2 gas exchange on BSCs.

Keywords: Comparison of chambers; cyanobacteria; infrared gas analyzer; lichens; mosses; net photosynthesis; soil respiration.

1. Introduction

In the context of increasing interest in more accurate local and global carbon balance measurements, many studies have analyzed the conditions under which different plant functional types, such as broadleaf and needle trees, shrubs and herbaceous C_3 and C_4 plants, are carbon sources or sinks (e.g., Cox et al., 2000; Sitch et al., 2003; Friedlingstein et al., 2006; Magnani et al., 2007). However, the relationship with carbon acquisition or loss by gas exchange of biological soil crusts (BSCs), defined as communities of cyanobacteria, algae, mosses, lichens and fungi in differing proportions, living on top of or within the first few millimeters of the soil surface, and closely bound to soil particles (Belnap et al., 2003), is still little known (Bowling et al., 2011). BSCs are especially important in the global CO_2 gas exchange in dryland ecosystems where they predominate and vascular plant productivity is limited (Lange et al., 1992; Lange, 2003). Indeed, there is evidence of their major contribution to soil respiration in semiarid ecosystems (Castillo-Monroy et al., 2011), as well as their photosynthetic capacity under appropriate moisture conditions (e.g., Brostoff et al., 2005; Li et al., 2012). Global net carbon uptake by cryptogamic ground cover has been estimated at about 2.5 Pg yr^{-1} (Elbert et al., 2012), which corresponds to around 4.5% of net primary production by terrestrial vegetation.

Infrared gas analyzers (IRGAs) have been used for decades to study CO_2 exchange in several types of organisms, such as vascular plants, cyanobacteria, mosses and algae (e.g., Bloom et al., 1980; Dring & Brown, 1982; Schipperges & Rydin, 1998; Brostoff et al., 2002; Millan-Almaraz et al., 2009). There are two main IRGA operating systems: open (i.e., where chamber air is renewed with ambient air from outside and CO_2 flux is calculated as the difference in CO_2 concentration ($[CO_2]$) between air in the chamber and incoming ambient air), and closed (i.e., where air is not renewed from outside the system and CO_2 flux is calculated as the change in chamber $[CO_2]$ during the sample enclosure time). Both systems have advantages and disadvantages, which can be maximized and minimized, respectively, depending on chamber design and configuration. The closed system was initially considered more accurate. It requires short measurement time, because, contrary to the open system, it does not need concentration gradient stabilization (Hutchinson & Mosier, 1981; Sebacher & Harriss, 1982; Mosier, 1989; Vourlitis et al., 1993). This becomes more advantageous as the

volume of the chamber increases (Hutchinson & Mosier, 1981; Livingston & Hutchinson, 1995). However, recently improved open system technology provides increased accuracy (LI-COR, 2003, 2012), whereas the main disadvantage of the closed system, the gradual alteration of the initial concentration of gases inside the chamber over time persists (Nakayama, 1990; Hutchinson et al., 2000; Davidson et al., 2002).

Although some studies have used chambers in closed systems (e.g., Kershaw, 1977; Snelgar et al., 1980), CO_2 fluxes in epiphytic, saxicolous and terricolous lichens have been usually measured with chambers coupled to open system IRGAs, in *ex situ* controlled environments (e.g., Carstairs & Oechel, 1978; Lange et al., 1986, 1992; Friedmann et al., 1993; Zaady et al., 2000). These *ex situ* studies have provided valuable information for interpreting the responses of lichens and BSCs in their natural habitats (Lange et al., 2001), but they have not shown how far the laboratory response measurements differ from *in situ* responses. For instance, they require samples to be removed from their underlying soil, and there is little knowledge on how BSC removal (and often thallus clipping) affects the functioning of the component organisms. Even in field work, when the BSC removed from the soil is measured immediately in a cuvette, problems may linger due to the disruption of substrate water supply (Schroeter et al., 1999). In addition, these studies usually have a physiological approach, focusing only on BSC gas exchange performance. However, in an ecological approach considering ecosystem implications, it is also necessary to take into account the contribution of the underlying soil, and therefore measurement of the whole soil profile becomes an advantage in quantifying the net ecosystem CO_2 balance. Despite these questions, there are few studies examining *in situ* net CO_2 exchange on biologically crusted soils, probably because the need for a more holistic approach to the subject has not come up until recent years.

As BSCs are very sensitive to humidity and temperature pulses (Grote et al., 2010; Bowling et al., 2011), reducing the enclosure time is critical to alter these two environmental factors as little as possible. Commercial canopy chambers which can be coupled to the soil surface, such as LI-COR and PP-Systems, cannot deal with this problem properly, as their volumes are not adapted to short measurement times. To overcome this drawback, we have designed a robust, easy-to-make chamber for short *in situ* measurements of net CO_2 gas exchange on undisturbed BSCs, and tested two versions with different diameters.

This matter is currently arousing considerable interest, as shown by the growing number of studies based on customized chambers to be coupled to the soil surface (Bremer & Ham, 2005; Botting & Fredeen, 2006; Langensiepen et al., 2012). However, their conclusions are unclear because their different methodologies are not fully comparable. Therefore, the aim of this study was to standardize the *in situ* gas exchange measurements on biologically crusted soils by (i) describing and discussing chamber design, and (ii) determining its specific measurement configurations by comparison with widely used commercial chambers. Specific steps for this purpose were to: (i) find the best combination of input airflow rate and fan speeds (internal system fan and additional fan inside the chamber if needed) as well as the best operation measurement system (closed or open); (ii) then with the best chamber configuration, verify the validity of the chamber design for measuring CO_2 fluxes by increasing the number of regression points in system comparisons and extending their ranges; and finally, (iii) examine any possible microenvironmental disturbances that could be caused by our chamber, especially keeping in mind that the BSC gas exchange is particularly sensitive to changes in temperature and humidity.

2. Materials and methods

2.1. Description of the chamber

An 8.5-cm-high transparent chamber was constructed from methacrylate tubes (supplied by *Rotufer S.L.,* Almería, Spain; and *Decorplax Metacrilatos S.L.,* Madrid, Spain). Most chambers used for this purpose are made of methacrylate because of its uniform 92% transmissivity at 400–800 nm wavelength light, and because it is lightweight, low-cost, shockproof and easy to handle and to bond with solvent glue (Bloom et al., 1980). However, its water adsorption is high, which is a problem if in addition to photosynthesis there is interest in evapotranspiration. Therefore, to avoid adsorption/desorption of water vapor, the inner chamber walls were covered with a Teflon® film, which is also highly transparent in the 300–900 nm range (Kesselmeier et al., 1996; Cocker et al., 2001). The top methacrylate surface was designed flat instead of the spherical shape used in other commercial chambers for easier manufacturing and handling in the field, and to reduce the measurement time due to the lower chamber

volume. The top can also be made with propafilm (provided by LI-COR Biosciences Inc., NE, USA), but we preferred a hard cover to make it more robust.

As an interface between the chamber and the IRGA (portable photosynthesis system LI-6400; LI-COR Biosciences Inc., NE, USA), we used a 9864-157 mounting plate (LI-COR Biosciences Inc., NE, USA) from a Conifer Chamber 6400-05. A flat frontpiece the same size as the metal plate was placed on the outside of the acrylic cylinder, and a methacrylate reinforcement was added inside the cylinder. A 0.5-cm-thick rubber gasket was added to the plate to seal the connection. We included an E-type thermocouple (not encapsulated) to record the surface temperature required for calculation of certain variables (such as vapor pressure deficit, total conductance to CO_2 and H_2O, or intercellular CO_2), as this temperature cannot be well estimated from air temperature with energy balance parameters in BSCs. An exhaust tube was inserted in a female hose fitting to route the air from the chamber to the match valve. The exhaust tube was placed at a 90°angle from the metal plate, far away enough to avoid shading. The chamber was designed to work on top of PVC collars previously inserted in the soil (see Castillo-Monroy et al., 2011 and Escolar et al., 2012 for this procedure). To ensure that the chamber was sealed on the soil, a toroidal disk covered with an air-tight rubberized band was placed between the chambers and the PVC collars inserted in the soil.

The use of different chamber diameters provides different possibilities, mainly related to the plot size required for each study target, whether almost isolated BSC species (small chamber) or BSC communities (large diameter chamber) are to be sampled. We therefore made two versions of the chamber with different diameters (Fig. 1), 10 and 20 cm (668 and 2,385 cm^3, respectively). The large chamber had an additional 5 x 5 cm^2 fan inside, also at a 90° angle from the metal plate (opposite the match exhaust tube), to minimize shading and optimize air circulation, and ensure that air entering the chamber was well mixed. It was powered by a 12V 7A battery connected to a voltage regulator so that the optimal fan speed could be selected. The holes for inserting the thermocouple and the fan cable in the chambers were sealed with Terostat-81®, which is neither gas source nor sink.

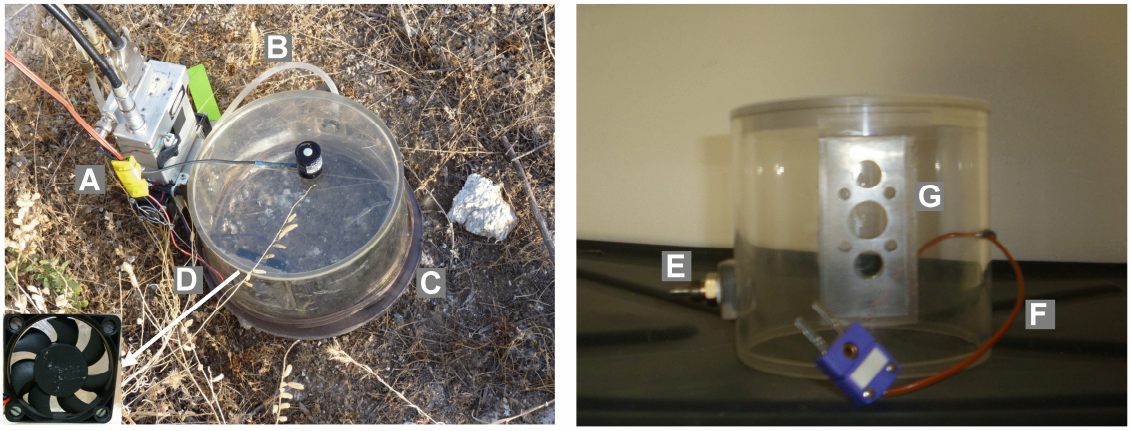

Figure 1 Left: large chamber with details of the fan voltage regulator (A); Exhaust tube (B); Toroidal disk (C); Additional fan (D), with a detailed in the bottom left-hand corner insert. Right: Small chamber with details of the female hose fitting (E); thermocouple (F); and the flat frontpiece with three large holes matching the mounting plate, and another four for the screws (G).

2.2. Comparison with commercial chambers using BSCs under laboratory conditions

As further precautions must be taken to operate with larger volumes (for example, measurement times and proper air mixing are more critical), we started the two comparative tests of this section on the larger chamber. The tests were also conducted in the small chamber to ensure that the selected configuration was appropriate for its volume, but with some abbreviations based on the previous results. As the core of these tests was the selection of the best chamber configuration for measuring on BSCs, they were performed under controlled laboratory conditions, using unaltered soil samples with three types of BSC: with a cover close to 100% of 1) *Diploschistes diacapsis* (Ach.) Lumbsch lichen, 2) *Cladonia convoluta* (Lam.) Anders lichen and 3) mosses (*Tortula* sp., *Crossidium* sp. and *Didymodon* sp.). PVC cylinder diameters of 10 cm (four samples for each crust type) and 20 cm (three replicates) were used to extract 6-cm-thick samples previously moistened to minimize damage to the crust. A brief description of the sampling sites can be found in the supplementary material.

Prior to measurements, both small and large samples were moistened with 60 and 200 ml of distilled water, respectively, in order to enhance the biological activity of the soil, and thereby gain a stronger respiratory signal. Measurements started at least 30 min after moistening to ensure steady CO_2 flux. LI-6400 system flow and IRGA CO_2 zeros were calibrated before each test, and when this device was used in open system

(Fig. 2), sample and reference IRGAs were matched before each measurement. During these measurements, we covered the transparent chambers with an opaque cloth to prevent photosynthetic activity, and CO_2 flux recorded in each sample was related to the measurement immediately following it by an opaque commercial chamber attached to the soil respirometer LI-8100 (LI-COR Biosciences Inc., NE, USA) or EGM-4 (PP-Systems, Hitchin, UK). The LI-8100 was used for comparison to the two prototype chambers because it has two chambers with the same diameters as ours (10 and 20 cm). Only the small chamber was compared to the EGM-4 respirometer, as this model only comes with a 10-cm-diameter chamber. Measurement time over the same sample was configured as 90 s, and data were stored every 5 s. The CO_2 flux measurements were adjusted to exponential and quadratic functions in the LI-8100 and EGM-4, respectively, to acquire the final sample flux. The use of a linear function is not recommended, as gas diffusion theory predicts that the rate of gas exchange across the soil-atmosphere interface will not be constant over the measurement period (Livingston et al., 2005). Absolute CO_2 flux was used for comparison between our chambers and commercial chambers, as the respiration and net photosynthesis systems have different signs.

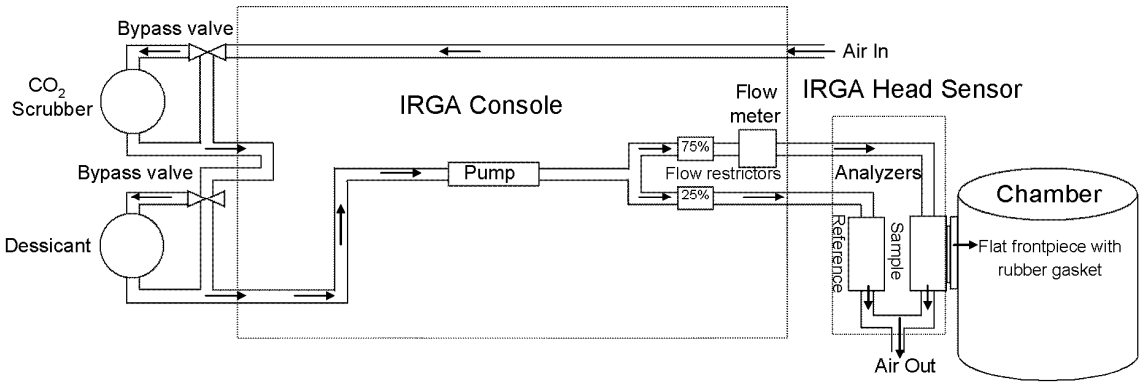

Figure 2. Schematic flows in an IRGA connected to our chamber, based on Li-6400/6400XT. The IRGA system can modify CO_2 and H_2O concentrations in the incoming air by means of chemical tubes (circles). The pump speed controls the flow.

2.2.1. Test 1.- Determination of the best airflow and additional ventilation speed

With the larger chamber, always operating as an open system and with the internal fan at its highest speed, two airflow rates (300 and 700 μmol s^{-1}) were combined with three air speeds provided by the additional fan (0.7, 1.1 and 1.4 m s^{-1}). When the best combination of fan speed and airflow rate was found, based on the best fit of these

measurements to the reference system (LI-8100), a last measurement was taken to check the effect of changing the speed of the sample IRGA's internal fan to low. Measurements were also performed without additional ventilation and with the best airflow rate previously found. In the small chamber, the airflow rate, additional and internal fan speed parameters were set according to the results of the large chamber comparisons, and the effect of additional ventilation was tested by comparing these chamber measurements, with and without fan, with those acquired by the EGM-4 reference chamber.

2.2.2. Test 2.- Open vs. *closed system configuration*

The respirometers used in this study operated in a closed system. In the IRGA attached to the prototype chambers (LI-6400), either open or closed system configurations may be selected. We tried both configurations with the samples, comparing their respiration responses measured by our custom chambers with those recorded by the LI-8100 respirometer. The open system was configured with the best input airflow rate and fan speed combination found in the first test. All measurements in this system were taken after the gas exchange inside the chamber had stabilized (i.e., the decimals in the photosynthetic rate were stable for one minute). However, when measuring in closed system, we used a procedure similar to that for the respirometers: the gas exchange rate was recorded every 5 s for 90 s, and the final rate was found by regressing the individual rates over time. The fan speeds (additional and internal) were the same as for open system, but in closed system air was not recirculated by the pump.

2.3. Outdoor comparison tests

We carried out another two tests with the best chamber configurations found in the section above. The aim of these tests was to extend the data range of the regressions by measuring net photosynthesis under natural light conditions using the commercial 6400-05 Conifer Chamber (LI-COR Biosciences Inc., NE, USA) as the reference system, and increasing the number of points in the regressions using both respiration devices for respiration measurements. The LI-6400 calibration protocol and configuration of the respirometers were the same as in the section above.

2.3.1. Test 3.- Net photosynthesis with our large chamber using vascular plants

As the 6400-05 Conifer Chamber is unsuitable for soil measurements, we compared the CO_2 fluxes measured by our large chamber (without the opaque cloth) with this commercial chamber, operated by another LI-6400 IRGA, using two vascular plants (*Ceratonia siliqua* L. and *Aspidistra* sp.). To close the custom chamber, a metal plate was placed on the bottom, and sealed with a gasket along the lower edge of the chamber wall. To eliminate the effect of any differences between the two LI-6400s in the comparison, both devices were previously compared using two of our large chamber prototypes with the same samples as in the preliminary comparison as well as with a vascular plant (*Aspidistra* sp.) to extend the measurement range. The small chamber was not used in this test.

2.3.2. Test 4.- Respiration with our chambers using ruderal and biologically crusted soils

Our large chamber was compared to the LI-8100 chamber under natural conditions. 16 PVC collars with a diameter of 20 cm were installed in the soil in an area with ruderal vegetation (weeds growing in human-disturbed fields). This soil was selected to widen the respiration range, as the previous crusted soils collected from semiarid ecosystems are characterized by low CO_2 efflux (Raich & Schlesinger, 1992; Rey et al., 2011). Herbaceous plants within the collars were removed, and soil respiration disturbance caused by collar installation was allowed to stabilize for 24 h. Half of the collar enclosures were left with their natural moisture, and the other half were moistened with increasing amounts of water from 100 to 1,000 ml to acquire a wide range of respiration. Measurement started after 12 h, alternating plots with and without moistening, first with the prototype chamber connected to the LI-6400, which was covered to ensure darkness like the opaque respirometer chamber, and then with the LI-8100 chamber. The measurements were carried out from 09:00 to 19:00 h (local time) in a total of six measurement cycles.

We compared our small chamber to an EGM-4 chamber under seminatural conditions, i.e., unaltered samples were *ex situ*, but measured outdoors. Samples of soil (12) with four kinds of BSC were collected, with three replicates for each type characterized by having a cover close to 100% of: 1) *D. Diacapsis* lichen, 2) *Squamarina lentigera* (Weber) Poelt lichen, 3) well-developed cyanobacterial BSC,

including pioneer lichens, and 4) incipient-cyanobacterial BSC. An analysis of the cyanobacterial community composition was not performed in this study, but based on a recent publication (Büdel et al., 2014), it is likely that *Microcoleus* sp. and *Nostoc* sp. were present in these samples. The measurements were taken for two days, the first with the samples watered to saturation, and the second with the remaining moisture after 24 hours of outdoor evaporation. Respiration on each sample (one cycle per day) was recorded with the prototype chamber connected to the LI-6400 (also covered with an opaque cloth), and immediately afterwards with the EGM-4 chamber.

2.4. Testing the influence of the chamber on micrometeorological variables

As a change in natural air pressure can directly affect gas diffusion in the soil-atmosphere interface (Kanemasu et al., 1974; Schlesinger, 1977), the relationship between airflow rate and/or additional ventilation and the pressure inside the large chamber must be carefully supervised. And, as mentioned above, BSCs are very sensitive to humidity and temperature micrometeorological variables, so any effect of the sample enclosure on these variables should also be checked.

2.4.1. Test 5.- Check for changes in air pressure inside the chamber

The pressure inside the large chamber was monitored under laboratory conditions by inserting an MSR 145 pressure sensor in the chamber. The threshold where pressure changes cause effects on BSC CO_2 measurements was also tested by generating an artificial overpressure inside the chamber. To cause this overpressure, an additional airflow introduced through the thermocouple orifice was provided by a bottle with 509.5 ppm CO_2 concentration. The CO_2 concentration of the flow generated by the LI-6400 was regulated by the 6400-01 CO_2 injector, and was the same as the extra airflow to avoid changes in the reference CO_2 concentration due to the use of two different flow sources. The enclosure effect in the small chamber was tested by selecting the best configuration found in the previous tests and without artificial overpressure.

2.4.2. Test 6.- Check for changes in air temperature and relative humidity (RH) inside the chambers

This test was performed in the large chamber under field conditions from dawn to noon on a summer day to cover wide natural temperature and solar radiation ranges. A DS1923 iButton® Temperature/Humidity Logger (Dallas Semiconductor, Texas, USA) was placed next to the large chamber and another one inside it, both directly over the BSC surface. Since soil water content on that test day was low (on average, 4% at a depth of 5 cm), and therefore, evaporation was also low, we used a series of measurements taken with the prototype chamber on several sunny days with the high soil water content (on average, 21% at a depth of 5 cm) as recorded on data loggers (model U23-001, HOBO Pro V2 Temp/RH Data Logger, Onset Computer Corporation, Bourne, MA, USA) placed 20 cm above the soil surface. In this case, the chamber air RH measured by the LI-6400 was compared with data logger records. The temperature test was not done in the small chamber, since the most influential factors in its change (material spectral properties and air renewal) were the same as in the larger one. RH changes in the small chamber were checked using the LI-6400 RH sample sensor records from Test 4 and environmental RH data from a nearby weather station provided by the Spanish Meteorological State Agency (AEMET).

3. Results

3.1. Test 1.- Determination of the best airflow and additional ventilation speed

The use of a low airflow caused flux measured in our large chamber was lower than the LI-8100 respirometer data, which was three-fold higher (Fig. 3). In all cases, the data acquired in our large chamber were lower than with the LI-8100 respirometer, but this difference could be reduced by raising the airflow to a ratio of 1.5:1. Increasing the speed of the additional fan worsened the correlation between the two CO_2 flux measurement systems. The configuration with a slow fan speed had the best R^2 using both high and low airflow rates. We selected a high airflow rate with slow fan speed as the optimum combination because the measurements were nearest the reference system, the R^2 was high, and the chamber stabilization time was half that required at the low airflow rate. Therefore, the effect of the sample IRGA mixing fan speed was tested only

with these optimal parameters. Best results were found with the IRGA fan at high speed (Table Supplementary material (S) 1; Fig. 4*A*), since although its regression was below the 1:1 line, their slopes were closer. We also found that, when the internal fan speed was lowered, R^2 fell from 0.83 to 0.59. The regression models fitted well with either low additional fan speed or without a fan, but with the first, LI-6400 data were slightly higher and closer to those of LI-8100 (Fig. 4*A*). An additional fan placed inside the small chamber and set at slow speed generally did not raise the measurements compared to the mode without ventilation (Fig. 4*B*), indicating that there were no problems of homogenization of the sample air in the absence of an additional fan.

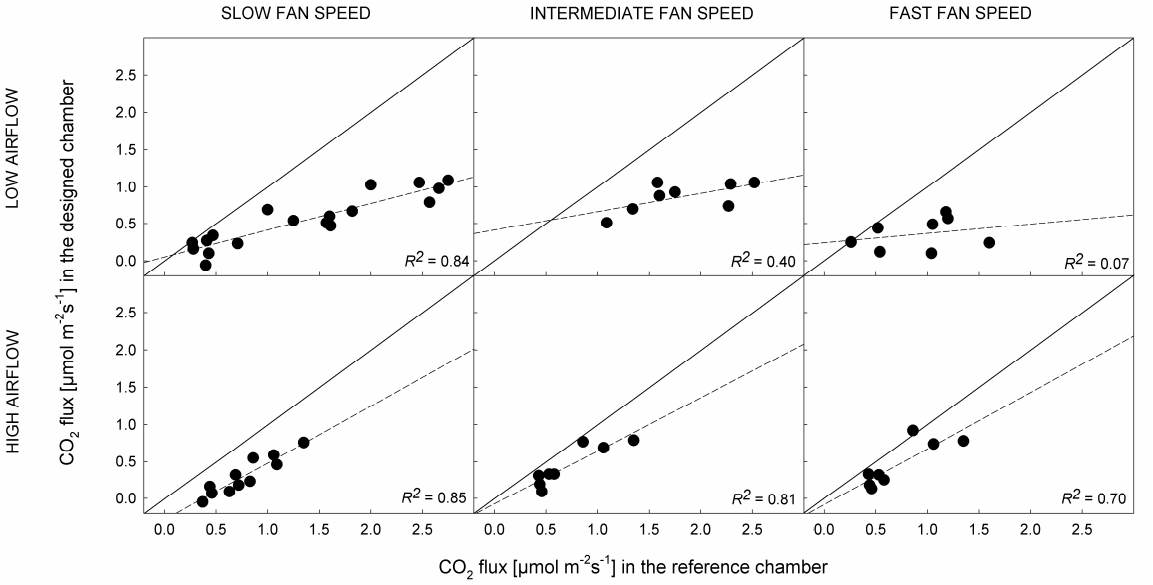

Figure 3 Relationship between the respiration measured by our large chamber, attached to an LI-6400, and the reference system (respirometer LI-8100), at different airflow rates and additional fan speeds. *Dashed lines* – linear regressions, *solid lines* – ideal 1:1 relationships. Airflow rates were: low = 300 µmol s^{-1} and high = 700 µmol s^{-1}. The fan speeds were: slow = 0.7 m s^{-1}, intermediate = 1.1 m s^{-1}, and fast = 1.4 m s^{-1}. The measurements were performed under laboratory conditions (≈ 20ºC, moistened BSC samples), and with the sample IRGA internal fan set at high speed.

3.2. Test2.- Open vs. closed system chamber configuration

In the large chamber, with the airflow and fan speed optimized, the coefficient of determination between the LI-6400 and the LI-8100 (respirometer) was substantially higher in open system than in closed system. In open system the regression slope was similar to the 1:1 line, although the CO_2 flow was underestimated (Fig. 4*C*). In the small

chamber, the R^2 coefficient was also higher in open than in closed system (Fig. 4*D*). Nevertheless, CO_2 records in both systems were lower and higher, respectively, than respirometer data.

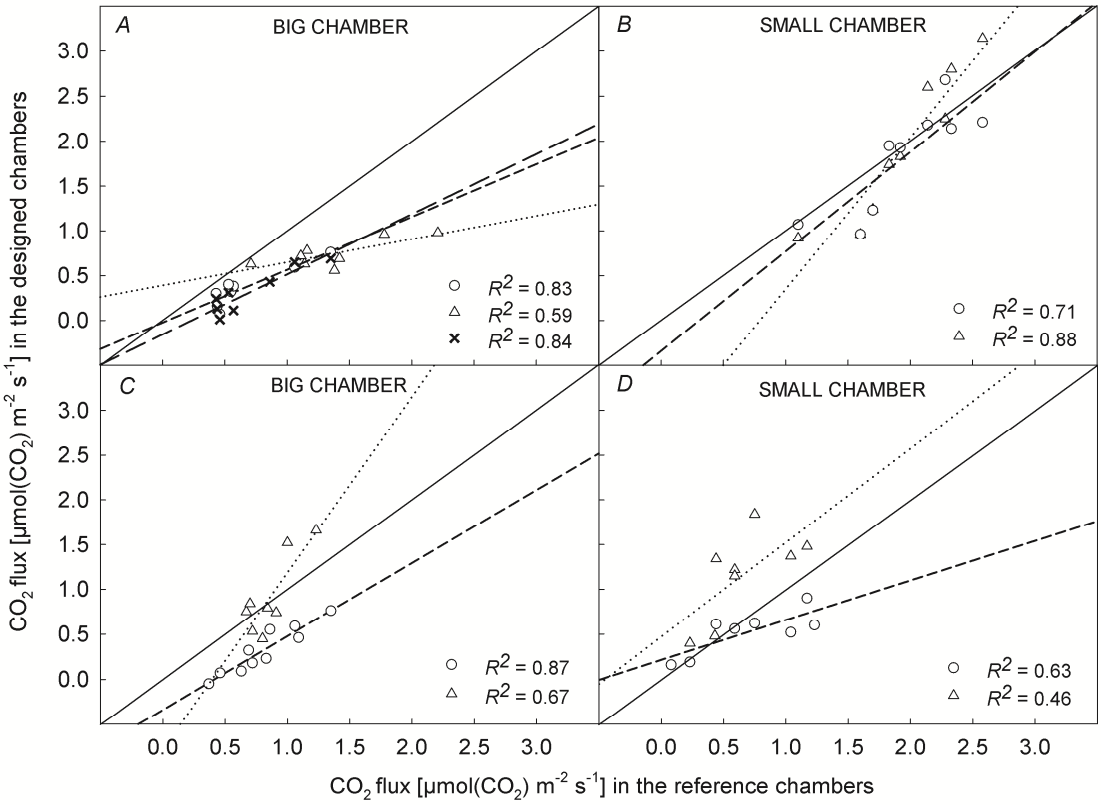

Figure 4 Comparison of our in-house designed chambers attached to an IRGA LI-6400, and commercial chambers (reference chambers), under laboratory conditions (≈ 20ºC, moistened BSC samples). Solid lines = ideal 1:1 relationships. *A*: our chamber operating in open system with the IRGA sample cell internal fan on high (circles and short dashed line) and low (triangles and dotted line) speed and the additional chamber fan on low (0.7 m s^{-1}). Measurements without the additional fan (crosses and long dashed line) and with the internal fan on high speed. *B*: the LI-6400 in open system with fan at low speed (circles and dashed line), without additional fan (triangles and dotted line), and internal fan on high speed. *C*: The LI-6400 in open (circles and dashed line) and closed (triangles and dotted line) systems, both with fan on slow speed. *D*: the LI-6400 in open (circles and dashed line) and closed systems (triangles and dotted line), both without fan. All open-mode measurements were done at an airflow rate of 700 µmol s^{-1}. The LI-8100 respirometer was the reference system in *A*, *C* and *D*, and the EGM-4 device in *B*.

3.3. Test 3.- Net photosynthesis measured with the large chamber vs. a conifer photosynthesis chamber

The large custom chamber measuring photosynthesis in the open system fitted well (R^2 = 0.959) to the commercial conifer chamber, with very similar measurements on the same samples, albeit slightly higher (Fig. 5*A*).

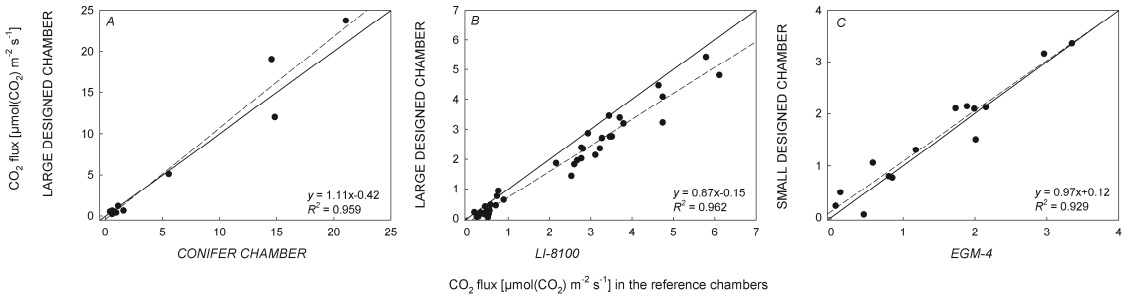

Figure 5 Comparison of our in-house designed chambers attached to an IRGA LI-6400, and commercial chambers (reference systems) at outdoor conditions. Dotted lines = Linear regressions; Solid lines = ideal 1:1 relationships. *A*: comparison in the net photosynthesis range with vascular plants. *B*: comparison of respiration in the range extended by using ruderal soil samples. *C*: comparison of respiration using BSC samples. Selected LI-6400 configurations: in *A* and *B*, IRGA sample cell internal fan at highest speed, additional fan at low speed (0.7 m s^{-1}), and airflow rate at 800 µmol s^{-1}; in *C*, IRGA sample cell internal fan at highest speed, without additional chamber fan, and airflow rate at 700 µmol s^{-1}.

3.4. Test 4.-Respiration under natural and seminatural conditions

The larger number of replicates and wider data range improved the fit between the two systems, resulting in a better correlation. The CO_2 flux in the LI-6400 continued to be lower than in the LI-8100, although less than in the preliminary tests (Fig. 5*B*).

As in the test with the large chamber, the increase in replicates and respiration range by using samples with different moisture levels over time improved the fit between our small custom chamber measurements and the EGM-4 respirometer (Fig. 5*C*). Measurements in both chambers were very similar even though the devices were from different manufacturers and operated in different configurations (open and closed systems).

3.5. Test 5.- Changes in air pressure inside the chambers

The pressure variation in the chambers did not exceed 150 Pa when the IRGA system operated with its own airflow system, and with the fan at low speed (large chamber) or without the additional fan (small chamber). Pressures ranged (including the initial pressure without closing the chambers) from 101.47×10^3 to 101.58×10^3 Pa in the larger chamber, and from 100.82×10^3 to 100.85×10^3 Pa in the small chamber. Thus, a high airflow in this system did not cause any pressure deficit or overpressure. When air from outside the system forced a pressure increase, the CO_2 flow remained unaltered as long as it did not exceed 200 Pa, but when it reached 300 Pa, net CO_2 flow rate increased (Fig. 6*A*). The variation in pressure at different additional fan speeds were far from achieving the threshold needed to alter natural CO_2 flow rates (Fig. 6*B*).

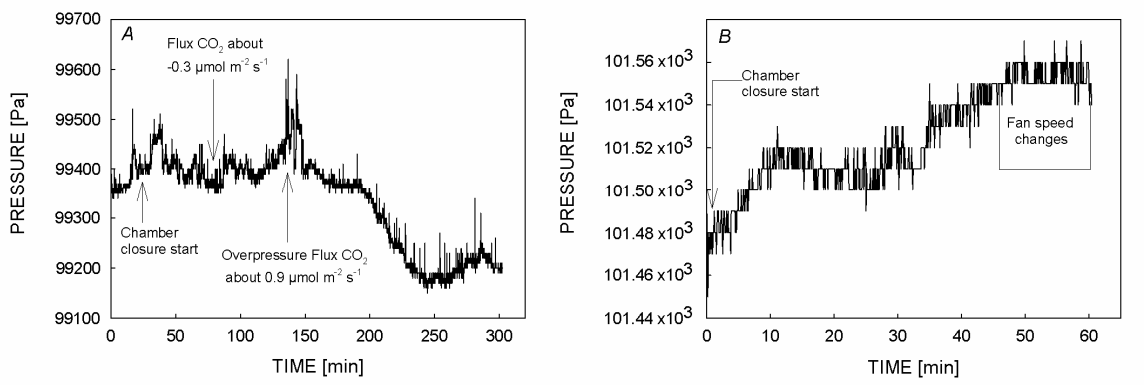

Figure 6 Air pressure test. *A*: Effect of increased pressure on CO_2 flux. Airflow rate in the LI-6400 800 µmol s^{-1}. *B*: Pressure in the large chamber during enclosure with changes in additional fan speed and 800 µmol s^{-1} airflow rate. Note that gas exchange is net assimilation: positive values imply a reduction in CO_2 concentration inside the chamber headspace, and negative values an increase.

3.6 Test 6.- Changes in temperature and RH inside the chambers

The paired measurements (outside-inside the chamber) showed that the air temperature inside the large chamber did not change with exposure to direct sunlight at temperatures below 30ºC, even though chamber closure time was over 4 min (Fig. 7*A*). However, when outside temperatures reached 30ºC, with the same closure time, the air temperature inside the chamber rose by as much as 5ºC. No relevant changes in air RH were observed inside the large chamber, where the average increase was only 3% in summer, late autumn and winter (Fig. 7*A*, *B*). However, in the small chamber, a considerable increase in RH was observed at high evaporation rates and when soil

moisture content was high, close to saturation: RH inside the chamber ranged from 37 to 94%, while the environmental RH only varied from 33 to 68%. See the supplementary material for a table summarizing all test results (Table S2).

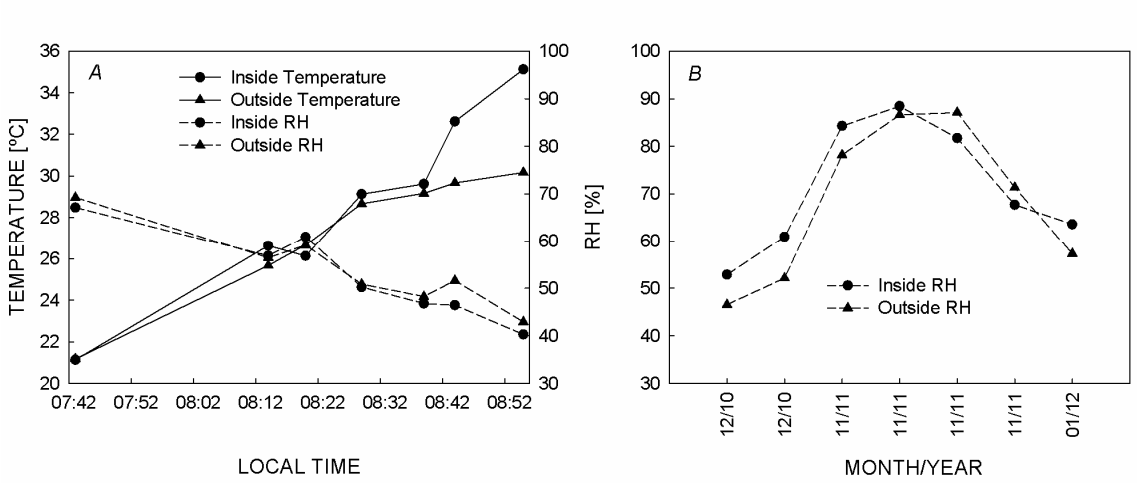

Figure 7 Temperature and RH test. *A*: Temperature and RH inside and outside the large chamber with stabilized CO_2 concentrations (on average in 4 min) measured on a sunny summer day. *B*: RH inside and outside of the large chamber on sunny days and with high soil water content.

4. Discussion

We have designed a versatile, robust, portable and transparent custom-built chamber for *in situ* net CO_2 exchange on soils covered by BSCs in two different sizes, and compared it with other systems. Internal air movement and system mode (open or closed) were configured to standardize their measurements with other commercial chambers. The configurations with the highest determination coefficients were considered the best, their gas exchange rates could be equated with those of commercial chambers by a correlation equation, and with minimal instrumental error.

The CO_2 exchange measured in our chambers for soil respiration or net photosynthesis in vascular plants is quite similar to the measurements with various widely used commercial chambers. The laboratory and seminatural conditions to which the biologically crusted soil samples were exposed can be compared in terms of temperature and moisture (the most important environmental variables for the soil respiratory activity) with the growing season in their areas of origin. The ranges of the reference systems in our tests carried out on BSCs (0.1–3.3 µmol CO_2 m^{-2} s^{-1}) were similar to those reported for *in situ* measurements during the growing season of one of

these areas. For example, Rey et al. (2011) measured soil respiration on the same type of soil as our samples, finding a range of 0.5 to 2.4 μmol CO_2 m^{-2} s^{-1}. On a soil moisture gradient from near 0 to 25%, and including different types of soil in this area, the respiration flux varied from 0.6 to 3.8 μmol CO_2 m^{-2} s^{-1} (Oyonarte et al., 2012). These ranges are lower than, for example, those found in subhumid forest ecosystems (e.g., Rey et al., 2002; Tang et al., 2009; Arevalo et al., 2010), but similar to those observed in semiarid regions (Chen et al., 2008; Almagro et al., 2009; Castillo-Monroy et al., 2011). Therefore, according to the reference systems, our chambers were configured within a range of naturally-occurring dryland fluxes.

We discuss the results of our comparative tests further below, in the context of the conclusions of several previous studies. Although the literature describing the design of chambers for measuring soil respiration is quite abundant, there is not as much on design for net photosynthesis. We also address some special considerations which must be taken into account for these measurements.

4.1. Additional ventilation and airflow configuration

The optimal CO_2 flux measurement was found with high air intake (700–800 μmol s^{-1}) and the additional fan at low speed (0.7 m s^{-1}), but with IRGA sample cell ventilation at high speed (Figs. 3, 5). Our additional fan at high speed caused the worsened correlation between the two systems, possibly due to the generation of turbulence causing increased measurement randomness. Other authors have also recommended the use of a low fan speed (e.g., Norman et al., 1997), as the turbulent airflows generated by high speeds decrease the soil boundary layer (Le Dantec et al., 1999; Janssens et al., 2000; Pumpanen et al., 2004). However, it is important to ensure adequate air mixing in the chamber. In the large chamber, the data recorded without ventilation were slightly lower than when moderate ventilation was used, showing that the additional fan in the chamber improved air mixing. In the small chamber, additional ventilation for proper air mixing was unnecessary, since data were no lower without fan action (Fig. 4*B*). In fact, the measurements with added ventilation had a lower R^2 and a stronger bias with respect to the EGM-4, possibly because the fan may have altered the aerodynamic conditions inside the chamber. This agrees with the LI-COR Biosciences Inc. recommendation that additional ventilation should be used when the volume of the chamber is over 2 L (LI-COR, 2003).

In open system, the steady-state CO_2 concentration gradient inside the chamber always differ from the outside natural conditions, but this disturbance can be minimized by optimizing the airflow generated by the system (Livingston & Hutchinson, 1995). Although high airflow rates may lower these gradient differences (Gao & Yates, 1998), they may also reduce measurement accuracy (LI-COR, 2003). However, according to our results, the highest LI-6400 airflow rate did not cause this problem with the chamber volumes employed, as demonstrated by their high correlation with the respirometers (Fig. 5). This was probably because the volumes were large enough in both cases to prevent the excessive air renewal that could cause CO_2 sample dilution to exceed sensor sensitivity levels (Ryden et al., 1978). Lower CO_2 fluxes when low airflow was used in our large chamber also reflected an air mixing problem. In addition, the high airflow rate shortened stabilization times (on average 2 and 4 min for the small and large chambers, respectively). This is especially important in poikilohydric organisms, for which enclosure times must be limited to a minimum due to the sensitivity of their metabolism to microenvironmental changes (Lange et al., 1997).

4.2. Comparison of systems under indoor conditions

We found a better correlation between the closed system LI-8100 and our chamber in open than in the closed system (Fig. 4*C*, *D*). CO_2 flux found with the LI-6400 in closed system was higher than in the open system. This was not consistent with the results found by Pumpanen et al. (2004) or Norman et al. (1997), who reported lower values in closed systems than in open systems. We do not think the lower CO_2 flux found in the open system was a result of poor air mixing, as we also found slightly lower values than with the LI-8100 at a high additional fan speed. In addition, we consider the basis for closed systems inadequate for measuring the response of photosynthesis in a wide range of environmental conditions, precisely because of the impossibility of maintaining those environmental conditions constant as a consequence of the absence of air renewal (Field et al., 1989; Gao & Yates, 1998). For example, in soil respiration, if the sample residence time is too long for a particular carbon efflux, the natural gas exchange in the soil-atmosphere interface decreases because of accumulation of the target gas in the chamber space (Rolston et al., 1976). This is one of the main problems of closed systems, and has been discussed in several articles (Ryden et al., 1978; Hutchinson &

Mosier 1981; Mosier, 1989; Rolston et al,. 1976; Nakayama, 1990; Freije & Bout 1991; Hutchinson et al., 2000; Davidson et al., 2002).

In fact, the open system has been used mainly for measuring photosynthesis in plants, whereas the closed system, cheaper and easier to handle, is used more frequently for soil gas fluxes (Healy et al., 1996; Conen & Smith, 2000). This is probably because stronger changes in CO_2 and H_2O concentrations inside the chamber in the closed system affect the physiology of plants (e.g., stomatal conductance and photosynthetic rates) more than soil respiration measurements. Thus, we conclude that the open system is the best operating mode for measuring net photosynthesis with our chambers.

4.3. Comparison of systems under outdoor conditions

The similarity of the LI-6400 (operating in the closed system) and the *LI-8100* soil respiration measurements has already been confirmed (Madsen et al., 2008). However, this study showed that this similarity continues to be valid when the LI-6400 is operated with our custom chambers in open system, considered a more appropriate method for measuring photosynthesis. Our two chambers were found to be consistent with the commercial chambers for both respiration and positive net photosynthesis over a wide range. The small chamber data were neither under nor over EGM-4 respirometer measurements. However, the large chamber data were slightly lower than the LI-8100 respirometer. Nevertheless, a high correlation between our chambers and the commercial respirometers and photosynthetic chamber measurements was found in all cases (Fig. 5, $R^2 > 0.9$), thus indicating the suitability of the custom chambers designed for measurement of CO_2 gas exchange on biologically crusted soils. Examples of its usefulness for measuring *in situ* net and gross photosynthesis can be found in Maestre *et al*. (2013) and in Chapter IV.

4.4. Changes in micrometeorological conditions inside the chamber

One of the main open-system biases in the measurement of CO_2 flux could be caused by the generation of pressure differences between the chamber space and the ambient due to air circulation inside the system (Rayment & Jarvis, 1997; Gao & Yates, 1998; Welles et al., 2001). Soil porosity enables CO_2 to escape from the soil, where it is much more concentrated than in the air (from 2,000 to 10,000 ppm in soil, compared to 350–

380 in the air, according to Welles et al., 2001). But, although CO_2 flux inside the chamber is less sensitive to overpressure than to pressure deficit, overpressure could lead to substantial underestimation of natural CO_2 flux (Fang & Moncrieff, 1998). This can be seen in Fig. 6*A*, where overpressure caused a positive net assimilation flux, which indicates a reduction of [CO_2] inside the chamber headspace. It should also be considered that both overestimation and underestimation of CO_2 flux are higher in dry soils (Hutchinson & Mosier, 1981; Lund et al., 1999), because water in soil prevents gas transport through the profile by advection, and greatly reduces molecular diffusion (Livingston & Hutchinson, 1995). It is therefore particularly important to consider whether the chamber operating configuration increases or decreases air pressure when used on dry soils.

No relevant pressure change was observed when measuring in open system (Fig. 6*B*), which shows that pressure deficit problems can be avoided by placing the pump before the air inlet into the chamber (such as in the LI-6400, see Fig. 2). But the most important factor that prevents important pressure changes is that the LI-6400 pump is underpowered for this. Although the optimal airflow found in this comparison was at the pump upper limit (1.34 L min^{-1}), according to Fang & Moncrieff (1996), this airflow hardly alters the atmospheric pressure inside the chamber, regardless of whether by suction or pumping, or the length and diameter of air inlet and outlet tubes. Moreover, we found that the use of additional small fans with slow speed in the large chamber did not cause any change in pressure.

Temperature rises inside the chambers because of the greenhouse effect of the material used, which can potentially directly and indirectly affect photosynthetic activity. Such increases change the affinity for O_2 of the primary carboxylating enzyme, RuBisCo, and increases evaporation from the soil surface, decreasing the magnitude and duration of BSC hydration (Grote et al., 2010). However, in our 4-minute-long field measurements, on sunny days, the temperature inside our custom chamber only increased when the outside temperature was over 25ºC. When the maximum outside temperature reached 30ºC, the temperature in the chamber was about 5ºC higher (Fig. 7*A*). The smaller temperature increase in this study may be due to ventilation and air renewal at a high airflow (800 µmols^{-1}), as the use of a static-closed chamber could cause an increase in air temperature of about 15ºC in only 3 min (Matthias et al., 1980).

Lichens with green algae as the photobiont have been found to be able to recover metabolic activity at high relative air humidity without any other source of water (Lange

et al., 1986, 1992). Thus, when soil evaporation is greater than the air renewal within the chamber, an increase in RH takes place in it, potentially causing artificial metabolic activation of some species in the BSC. Chamber measurements may thus overestimate the photosynthesis of poikilohydric organisms, as found by Wilske et al. (2008) in field measurements on BSC. It is therefore important to control RH when small chamber volumes are used. In our large chamber, no relevant changes in RH were observed due to the stronger air recirculation, and to the chamber volume, which acted as a buffer (Fig. 7*A*, *B*). However, under high evapotranspiration conditions, the small chamber was close to air water vapor saturation. The humidity input in the small chamber therefore needs to be regulated under extremely high moisture content and solar radiation conditions, which can be achieved by turning the LI-6400 desiccant adjustment screw to scrub position, keeping H_2O vapor from entering the circuit (see Fig. 2).

5. Conclusions and usefulness of the chamber

Our chamber, measuring in open system mode, at high airflows and with the sample IRGA mixing fan at the highest speed, was demonstrated to be very suitable for measuring CO_2 exchange on soils with BSCs in a wide range of both positive and negative values. Custom manufactured chambers such as those proposed here, have five main advantages: (i) they are cheaper than commercial chambers; (ii) chamber diameters can be adapted to the best plot size for the study target (isolated species or communities); (iii) the chamber volume can be minimized for faster measurement and fewer changes in the environment near the sample surface; (iv) due to the shortened measurement time, more complete experiments with larger sample sizes can be designed; (v) and furthermore, our chamber can also be used as a respirometer simply by covering it with an opaque cloth. On the other hand, different chamber sizes also require different precautions for their proper application. For example, our larger chamber has to have additional low-speed ventilation for adequate air sample homogenization; and as our smaller chamber is more sensitive to changes in humidity, it requires manual adjustment of the humidity input for measuring under a high soil water content and evapotranspiration demand. Our results also demonstrate that this methodology is consistent and appropriate for use under field conditions, and the good

correlations found with commercial respirometers mean that, for example, these two systems may be used simultaneously to estimate gross photosynthesis.

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SUPPLEMENTARY MATERIAL

Easy-to-make portable chamber for *in situ* CO_2 exchange measurements on biological soil crusts

Journal: Photosynthetica

M. Ladrón de Guevara, R. Lázaro, J.L. Quero, S. Chamizo and F. Domingo

Sampling sites

Figure S1 Indoor configuration tests: soil samples were collected in the Balsa Blanca instrumented area (36º 56'N - 2° 02'W, Almería, Spain), located in the Cabo de Gata Natural Park. This site is representative of a Mediterranean steppe ecosystem, with vegetation dominated by *Macrochloa tenacissima* (alpha grass), a perennial tussock grass, and open areas of bare soil, stones and biological soil crust.

Figure S2 Outdoor comparison test in the small chamber: soil samples were collected in El Cautivo instrumented area (37º 00'N – 2º 26'W, Almería, Spain). The area is a thermo-Mediterranean semiarid ecosystem, located in one of the most extensive badlands in Spain, where many types of biological soil crusts show extensive development, as well as physical crusts.

Table S1 Linear Regressions and F ANOVA for the various chamber configurations tested. Numbers in bold show significant differences ($P < 0.05$). Statistical analyses were performed using SPSS.

Configuration	R^2	F	*P*
Big chamber: airflow and fan parameters test			
Low airflow and slow fan speed	0.836	81.344	**0.000**
Low airflow and intermediate fan speed	0.398	3.970	0.093
Low airflow and fast fan speed	0.070	0.451	0.527
High airflow and slow fan speed	0.849	50.766	**0.000**
High airflow and intermediate fan speed	0.810	25.515	**0.002**
High airflow and fast fan speed	0.712	14.834	**0.008**
High airflow without fan	0.839	31.359	**0.001**
Sample IRGA mixing fan in low position	0.585	9.872	**0.016**
Sample IRGA mixing fan in fast position	0.830	29.344	**0.002**
Big chamber: system modes comparison			
Open system	0.861	49.625	**0.000**
Closed system	0.688	13.253	**0.011**
Small chamber: fan parameter test			
Slow fan speed	0.711	17.228	**0.004**
Without fan	0.877	49.811	**0.000**
Small chamber: system modes comparison			
Open system	0.631	10.254	**0.019**
Closed system	0.464	5.204	0.063

Table S2 Summary table of the test procedures carried out. Numbers denoted custom chambers vs.: (1) LI-8100; (2) EGM-4; (3) LI-6400 conifer chamber.

Tests	668 cm^3 custom chamber	2385 cm^3 custom chamber
Preliminary best configuration options		
Test 1: airflow	700-800 μmol s^{-1} (2)	700-800 μmol s^{-1} (1)
Test 1: additional fan speed	Not recommended (2)	0.7 m s^{-1} (1)
Test 1: internal fan speed	LI-6400 high speed option (2)	LI-6400 high speed option (1)
Test 2: system mode	Open system (1)	Open system (1)
Improved comparison tests		
Test 3: net photosynthesis measurements	Not implemented	R^2 = 959; slight overestimation (3)
Test 4: respiration measurements	R^2 = 0.929; 1:1 relationship (2)	R^2 = 0.962; slight underestimation (1)
Testing the influence of the chamber on micrometeorological variables		
Test 5: pressure changes	Not implemented	Not relevant with the selected settings
Test 6: temperature (T) and RH changes	T no implemented; RH rises in some circumstances	Maximum increase of 5°C; Not relevant RH increase

Chapter IV

Simulated climate change reduced the capacity of lichen-dominated biocrusts to act as carbon sinks in two semi-arid Mediterranean ecosystems

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SIMULATED CLIMATE CHANGE REDUCED THE CAPACITY OF LICHEN-DOMINATED BIOCRUSTS TO ACT AS CARBON SINKS IN TWO SEMI-ARID MEDITERRANEAN ECOSYSTEMS

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Abbreviations: NRWI, non-rainfall water inputs; biocrust, biological soil crust; Crust, biocrust cover; WA, warming; RE, rainfall exclusion; F_v/F_m, maximum photochemical efficiency of photosystem II. IRGA, infrared gas analyzer; RS, passive rainout shelters; OTC, open top chamber; PAR, photosynthetically active radiation; RH, air relative humidity.

Abstract

The importance of biological soil crusts (biocrusts) for the biogeochemistry of drylands is widely recognized. However, there are significant gaps in our knowledge about how climate change will affect these organisms and the processes depending on them. We conducted a manipulative full factorial experiment in two representative dryland ecosystems from central (Aranjuez) and southeastern (Sorbas) Spain to evaluate how precipitation, temperature and biocrust cover affected the assimilation and net carbon balance of biocrusts. Chlorophyll fluorescence, net photosynthesis and dark respiration were measured *in situ* bimonthly during a year. We also conducted daily cycle measurements of net photosynthesis in winter and at the end of spring. In Sorbas, warming reduced the fixation of atmospheric carbon in biocrust-dominated microsites throughout the year. In Aranjuez, there was an interaction between the three factors evaluated; during winter, net photosynthesis was significantly greater in high biocrust cover plots under natural conditions and in the rainfall exclusion treatment. During the daily surveys, rainfall exclusion and warming reduced carbon fixation in Sorbas and in Aranjuez respectively. The effects of the treatments evaluated varied with the rainfall and non-rainfall water inputs (NRWIs) registered before the measurements. Our results suggest that changes in NRWI regimes as consequence of warming could have a greater impact on the carbon balance of biocrusts than changes in rainfall amounts. They also indicate that climate change may reduce the photosynthetic ability of lichens, with a consequent reduction of their dominance in biocrust communities at the mid to long term. This could reduce the ability of dryland ecosystems to fix atmospheric carbon.

Keywords: warming; rain exclusion; lichens; gas exchange; chlorophyll fluorescence; semi-arid land.

1. Introduction

In drylands, plant interspaces are usually covered by biological soil crusts (hereafter biocrusts), communities composed of bacteria, fungi, algae, lichens, liverworts and mosses (Eldridge & Greene, 1994; Belnap, 2003; Maestre et al., 2011). The importance of biocrusts for biogeochemical cycles and hydrogeomorphological processes in drylands is widely recognized (reviewed by Belnap, 2003; Belnap & Lange, 2003; Belnap, 2006). While photosynthetic organisms forming biocrusts have lower net photosyntetic rates than vascular plants, in areas where plants are scarce or physiologically limited, biocrusts are considered a major component of the CO_2 uptake by the whole ecosystem (Lange et al., 1992; Garcia-Pichel & Belnap, 1996; Lange, 2003). Indeed, and at global scale, a carbon net assimilation of ~ 2.5 Pg a^{-1} by cryptogamic ground covers has been estimated, corresponding to around 4.5% of net primary production by terrestial vegetation (Elbert et al. 2012).

Biocrust-forming organisms typically show a fast metabolic response to changes in their microenvironmental conditions, such as water scarcity, high and low temperatures and elevated light exposure (Lange et al., 1997, 1998; Kappen et al., 1998; Kranner et al., 2003; Zheng et al., 2011). Since biocrust constituents occupy the soil surface (usually with higher temperatures than the air during cloudless days), and lack stomatal control, they dry out quickly (Bowling et al., 2011). Hence, they are very sensitive to humidity and temperature pulses, these being the main factors determining their biological activity in drylands (Veste et al., 2001; Grote et al., 2010; Bowling et al., 2011). Most previous studies on the photosynthetic behaviour of biocrust constituents have been conducted in the laboratory (Lange et al., 2001; Lange, 2003; Grote et al., 2010), or have separated them from their underlying soil, with the consequent possibility of having eliminated the major factor influencing their CO_2 exchange (Wilske et al., 2008). There are also important uncertainities about how the metabolic activity of biocrusts will be affected by predicted changes in climate, and how climate change-induced effects on biocrusts will impact the carbon balance of drylands (Maestre et al., 2013). Therefore, more information about the *in situ* gas exchange of unaltered biocrusts, as well as a better understanding of the relationship between environmental factors and the photosynthetic activity of biocrusts, is necessary to improve our ability to predict how climate change will impact drylands.

Organisms such as lichens may also be suitable model organisms to detect changes in photosynthetic activity as a result of climate change because of their lack of homeostatic mechanisms to cope with extreme environmental conditions (Lechowicz, 1982). This fact is important for the new generation of climate models, which consider the feedbacks between vegetation dynamics and climate change (e.g., Randerson et al., 2009; Thornton et al., 2009; Friedlingstein & Prentice, 2010). To improve our understanding on how climate change will impact the physiological activity of biocrusts, we conducted a full factorial climate manipulative experiment at two field sites in Spain. Based on previous studies that higlighted the importance of wetting events in the carbon balance of poikilohidric organisms (e.g., Čabrajić, 2009; Reed et al., 2012), and the negative effects that temperature increases have on maintaining soil moisture (e.g., Grote et al., 2010; Su et al., 2012), we hypothesized that predicted changes in climate will have a negative effect in the ability of biocrusts to fix carbon (Maestre et al., 2013), and thus in the capacity of dryland ecosystems to be carbon sinks. Our objectives were to: (i) explore the main temporal features of the biocrust carbon fixation ability on daily and seasonal scale, and how they relate to climatic variables; (ii) evaluate how predicted changes in climate (warming of 2–3ºC and ~ 30% reduction in rainfall; Escolar et al. 2012) will affect the *in situ* photosynthetic efficiency and gas exchange of biocrusts; and (iii) verify whether climate change effects on these variables vary with the degree of biocrust development.

2. Materials and methods

2.1. Study sites

The study was conducted at two research sites: Aranjuez (40º 02'N – 3º 32'W; 590 m a.s.l.) and Sorbas (37º 05'N – 2º 04'W; 397 m a.s.l.), located in central and south-eastern Spain, respectively. Their climate is Mediterranean semi-arid, with a mean annual temperature and rainfall of 15ºC and 349 mm, and 17ºC and 274 mm in Aranjuez and Sorbas respectively. Both sites are Miocene gypsum outcrops, with soils classified as and are classified as Gypsiric Leptosols (IUSS Working Group WRB, 2006). The cover of perennial vegetation is below 40% at both study sites, and is constituted mainly by the tussock grass *Macrochloa tenacissima* (= *Stipa tenacissima*, alpha grass). Some shrubs or dwarf shrubs (e.g., *Retama sphaerocarpa*, *Gypsophyla*

struthium, Helianthemun squamatum), as well as some crass perennial plants (e.g., *Sedum gypsicola, Sedum sediforme*), are also common to both areas. Over half of the plant interspaces are colonized by a well-developed biocrusts dominated by green algae lichens in both sites, being the most abundant *Diploschistes diacapsis, Squamarina lentigera, Cladonia convoluta, Fulgensia spp.* and *Psora decipiens* (see Maestre et al., 2013 for a species checklist).

2.2. Experimental design

A full factorial manipulative experiment was setup in July 2008 and May 2010 in Aranjuez and Sorbas, respectively. The factors, all of them with two levels, were: (i) biocrusts cover (Crust; poorly developed biocrusts communities with cover < 25% vs. well developed biocrusts communities with cover > 50%); (ii) warming (WA; natural vs. 2–3ºC temperature increase); (ii) and rainfall exclusion (RE; natural vs. ~ 30% rainfall exclusion). Throughout the text we refer to the control treatment as the natural temperature and rainfall conditions. The number of replicates was 10 and 8 in Aranjuez and Sorbas, resulting in a total of 80 and 64 experimental plots at these sites, respectively. A minimum separation distance between plots of 1 m was established to minimize the risk of sampling non-independent areas. The plots were 1 m^2 with a PVC collar of 20 cm in diameter in their centers for biocrust monitoring. The collars were inserted approximately 5–6 cm into the soil, standing out 2–3 cm over it.

The warming treatment aimed to simulate the average of predictions derived from six Atmosphere-Ocean General Circulation Models for the second half of the 21st century (2040-2070) in central and Southeast Spain, which predict increases of 2.1–2.3ºC during winter months, and 3.2–3.5ºC during summer months (De Castro et al., 2005). To achieve a temperature increase within this range, we used open top chambers (OTCs) of hexagonal design with sloping sides of 40 cm × 50 cm × 32 cm (see Escolar et al., 2012 for details). These structures were built with methacrylate, and were elevated between 3 and 8 cm from the soil to allow air circulation over the surface of the plots, and thus avoid an excessive overheating.

Predicted changes in rainfall for our study area are subjected to a high degree of uncertainty, but most climate models forecast important reductions -between 10% and 50%- in the amount of spring and fall rainfall (De Castro et al., 2005). To simulate these conditions, we set up passive rainout shelters (RSs) based upon the design described by

Yahdjian & Sala (2002). Each RS has an area of 1.44 m^2 (1.2 m × 1.2 m), a mean height of 1 m, and is composed of three methacrylate grooves, which cover approximately 37% of the surface, connected to plastic bottles that accumulate the excluded water in order to control it. See Escolar et al. (2012) and Maestre et al. (2013) for additional details of the experimental installations and their effects on microclimatic variables.

2.3. Microclimate monitoring

The effects of the OTCs and RSs on air temperature and relative humidity (RH) were continuously monitored using automated sensors (HOBO U23 Pro v.2 Temp/RH, Onset Corporation, Bourne, MA, USA), and those on soil surface (0–2 cm) temperature and moisture (0–5 cm depth) were continuously monitored on the two levels of Crust factor using TMC20-HD and EC-5 sensors, respectively (Onset Corp. and Decagon, Inc., Pullman, WA, USA). Soil moisture (0–5 cm depth) was measured discontinuously with time-domain reflectometers (TDR, Topp and Davis, 1985). To compare these effects between treatments, air and soil temperatures were calculated as the temperature average during the gas exchange measurement period, and soil moisture measured discontinuously was preferably used.

Two sources of non-rainfall water input (NRWI) were studied: dew and fog. Several studies have found a positive and significant relationship between the duration of these events and the amount of water condensed on the surface (e.g., Zangvil, 1996; Beysens et al., 2005; Kidron, 2000; Uclés et al., 2013a). But the main advantage of detecting these events lies in providing information on the duration of their occurrence, which could even be more important than their amount regarding biological activity and species distribution (Malek et al., 1999; Kidron & Temina, 2013). Hence, dew and fog condensations were studied in terms of their durations, and the NRWI between plots with and without OTC was compared based on this assumption.

Dew occurs when the temperature of the surface where water condenses is equal or lower than the dew point of the surrounding air. Measuring the temperature of the soil surface with the enough accuracy to determinate dew with *in situ* sensors is difficult, but previous studies have done so using thermocouples buried on the soil (Uclés et al., 2013a, b). Hence, we estimated the duration of dew as the time in which the surface temperatures of the sensors TMC20-HD were below the dew point

temperature of the air (this being calculated from the temperature and RH measured by the HOBO U23 sensors).

Fog is produced when the atmospheric water vapour concentration reaches saturation and condensed water droplets remain suspended on the air. In this study, the occurrence of fog events was considered when relative air humidity was higher than 98.5%, as the sensors typically have an accuracy error of ±2.5% (Onset Corporation, 2013).

2.4. Gas exchange measurements

The CO_2 exchange of the whole soil column, including the biocrusts living on its surface, was measured at six randomly chosen replicates per combination of treatments every two months between September 2010 and January 2012. These measurements were conducted at both study sites after dawn, starting when the collars received direct light, and always on cloudless days to avoid the interference of occasional clouds in the response of any treatment, or hour in the daily cycles. This is a representative meteorological situation of the experimental areas as, on average, Aranjuez and Sorbas have 94 and 106 cloudless days per year, respectively; AEMET, 2013). Previous studies have found that, in drylands with relevant water supply as dew or fog, such as those we studied, the photosynthetic activity of biocrust constituents peaks during early morning hours in the absence of rain (Kappen, 1988; Lange et al., 1992, 2006; Veste et al., 2001). In addition to the absence of clouds, we performed all measurements within a maximum interval of two hours to maintain homogeneous environmental conditions, so half of the replicates were measured in one day, and the other half were measured on the next day.

We measured net photosynthesis (µmol CO_2 m^{-2} s^{-1}) using two infrared gas analyzer (IRGA) systems (LI-6400, Lincoln, NE, USA) connected to a customized methacrylate transparent chamber with a volume of 2385 cm^3 (see Chapter III). To ensure the sealing of the chamber on the sample, a toroidal flat disk covered with an air-tight rubberized band was placed between the chamber and the PVC collar. The devices were operated as open dynamic systems, i.e., chamber air was renewed with a continuous external air flow and CO_2 flux was calculated as the difference in [CO_2] between chamber air (measured by an IRGA referred to as sample) and external input air (measured by an IRGA referred to as reference). The external input air was taken at

two meters above the soil surface by a rod to use a natural atmospheric air without the influence of the biological activity (photosynthesis and respiration of soil and plants) and the operator's breathing on its [CO_2], and a bottle of 6 L was used as a buffer volume in order to use an input air with a stable [CO_2]. The external air circulated through the system with a flow of 800 μmol s^{-1}, and an additional ventilation into the chamber of 0.7 m s^{-1} were used to obtain an adequate air mixing. System flow and IRGA CO_2 zeros were calibrated each day before measurements, and H_2O zeros were calibrated the first of the two consecutive measurement days of each bimonthly data collection. Before each individual measurement we matched the CO_2 readings of the sample and reference IRGAs, as this is very important for the accuracy of the measurements when low ΔCO_2 values are expected (LI-COR, 2012). The net photosynthesis value of a plot was recorded when the CO_2 values of the sample and reference IRGAs were stabilized.

Photosynthetically active radiation (PAR) was measured with a LI-COR external quantum sensor (9901-013, LI-COR, Lincoln, NE, USA), and with a Field Scout Quantum Light Meter (Spectrum Technologies, Plainfield, IL, USA), both of them placed at the top of the transparent chamber to have a reference value close to the biocrust surface. It was recorded at the same time of the net photosynthetic activity, and was used to consider in the statistical analyzes a potential source of variability in photosynthesis values because of environmental changes unrelated to our treatment. Immediately after these records, dark respiration was measured in the same plots with two soil respirometers (LI-8100, Lincoln, NE, USA). They operated as closed dynamic systems, i.e., there was a continuous recirculation of air inside the system, but without being renewed with an outside air intake. CO_2 flux was calculated as the change in chamber [CO_2] during the sample enclosure time adjusted to an exponential function. Each respiration measurement lasted 120 s, this being the recommended time when is expected the low flux characteristic of dryland ecosystems (Castillo-Monroy et al., 2011; Rey et al., 2011). A fitting equation between these two systems (LI-6400 and LI-8100) was performed covering the clear chamber attached to LI-6400 with an opaque cloth (R^2 = 0.96), and it was applied on the dark respiration records. As both devices measured gas exchange of the whole soil profile, in both cases the respiration of autotrophic and heterotrophic components was present, and gross photosynthesis could be calculated as the sum of net photosynthesis and soil dark respiration.

Daily cycles of net photosynthesis were also conducted in May 2010 in Aranjuez, and in January 2012 at both study sites. They were performed with the same protocol described above, but the measurements were extended throughout the daylight, and only collars with well-developed biocrusts were measured. During the measurements conducted in May 2010 only net photosynthesis was measured, but in January 2012 net photosynthesis (Li-6400) and dark respiration (LI-8100) were taken, so we could estimate gross photosynthesis.

2.5. Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured by computing the parameter $F_v/F_{m,}$, an indicator of the status of the photosystem II in photosynthetic organisms (Maxwell & Johnson, 2000), with a Handy PEA fluorometer (Hansatech instruments, Kings Lynn, UK). Low values indicate the presence of physiological stress (Maxwell & Johnson, 2000). These measurements were conducted simultaneously with the bimonthly gas exchange measurements described above, but only in the Sorbas site. F_v/F_m was calculated as the ratio between the variable (F_v = F_m-F_o) and the maximum (F_m) fluorescence signal. Prior to measurements, lichens were dark adapted using dark cloth for 30 min. Fluorometer measurements of the whole biocrusts community were made in four replicated plots per combination of treatments. Six random measurements per plot were taken in all cases, which were averaged for further analyses.

2.6. Data analyses

We evaluated the effects of biocrust cover (Crust), warming (WA), rain exclusion (RE), and their interactions, on the measured variables (net and gross photosynthesis, dark respiration and F_v/F_m) over time using general linear mixed models (LMMs) by means linear mixed-effects models (lme) procedure from Linear and Nonlinear Mixed Effects Models (nlme) R package (Pinheiro et al. 2013). The generic function of lme fits a linear mixed-effects model in the formulation described in Laird & Ware (1982), but besides allowing for nested random effects (Pinheiro et al., 2013). LMMs expand on the ordinary linear regression model by allowing one to incorporate lack of independence between observations and to model more than a single error term (Cnaan et al., 1997),

so temporal correlated errors and variance of the errors can be modeled in order to avoid pseudoreplication and heteroscedasticity problems, respectively.

Four fixed factors were established in the bimonthly survey analyses: measurement date, Crust, WA and RE. All their possible interactions were included in the models. In daily cycle analyses, the Crust factor was not applied, as only high biocrust cover plots (> 50%) were measured. The analyses were implemented in InfoStat software version 2013p (Di Rienzo et al., 2013), a friendly interface to use R (version 2.15.2). The restricted maximum likelihood (REML) procedure, which takes into account the loss in degrees of freedom resulting from estimating fixed effects (Patterson & Thompson, 1971), was used to estimate the variance and covariance components. The best structures for our models were selected with the AIC criterion (Akaike, 1973). For the analyses of net and gross photosynthesis, we also evaluated wheter the inclusion of PAR as a covariate in the models improved their fit. Fisher's least significant difference (LSD) post-hoc test was applied on the most complex interactions of the factors that showed significant differences. The effect of WA on NRWI duration was analyzed for each measurement day using t-tests. When the variance was not homogeneous, we used the non parametric Mann-Whitney test instead.

3. Results

3.1. Treatment effects on micrometeorological conditions during measurements

Summer drought was more acute in Sorbas than in Aranjuez (Fig. 1). The distribution of rainfall events was also less equitable in Sorbas site. However, the amount of rainfall registered in the days prior to the measurements was greater in Sorbas, especially in December 2010, September 2011, and November 2011. The winter 2011–2012 was characterized by an unusually low number of rainfall events in Aranjuez. Nighttimes preceeding the measurements were characterized by the formation of dew and fog in Aranjuez and Sorbas, particularly during winter. Dew formation and small events of fog predominated in Sorbas, whereas long duration fog events (more than 4 h) were more common in Aranjuez. Warming reduced the duration of dew and fog at all events, albeit significant differences were only found in short events (two in Sorbas, and another two in Aranjuez; Fig. 1).

During the bimonthly measurements, the WA + RE treatment had consistently the lowest soil moisture values in Sorbas (Fig. Supplementary material (S) 1a, b), whereas in Aranjuez the treatment with such values varied through time and with the degree of biocrust cover (Fig. S1c, d). OTCs caused a soil temperature increase of 1.7ºC and 3.3ºC on average in Sorbas and Aranjuez, respectively (Fig. S2a, b). In Sorbas, this treatment also increased early morning air temperature 2.4°C in February and 5.6ºC in April (Fig. S2c). In Aranjuez (Fig. S2d), this increase was over 2ºC only in February (2.4ºC), and was lower or did not occur in the rest of dates at both sites, as the temperature differences in plots with and without OTCs were between -1.8 and 1.6ºC. During the January daily cycles (Fig. 2a, b), OTCs caused a maximal increase in air and soil temperatures of 4ºC and 3.3ºC, respectively, in Sorbas, and of 4.3ºC and 3.5ºC in Aranjuez. In May (Fig. 2c), the maximal increase in air temperature in Aranjuez was greater (6.8ºC), but increases in soil temperature were similar to those recorded in winter (3.3ºC).

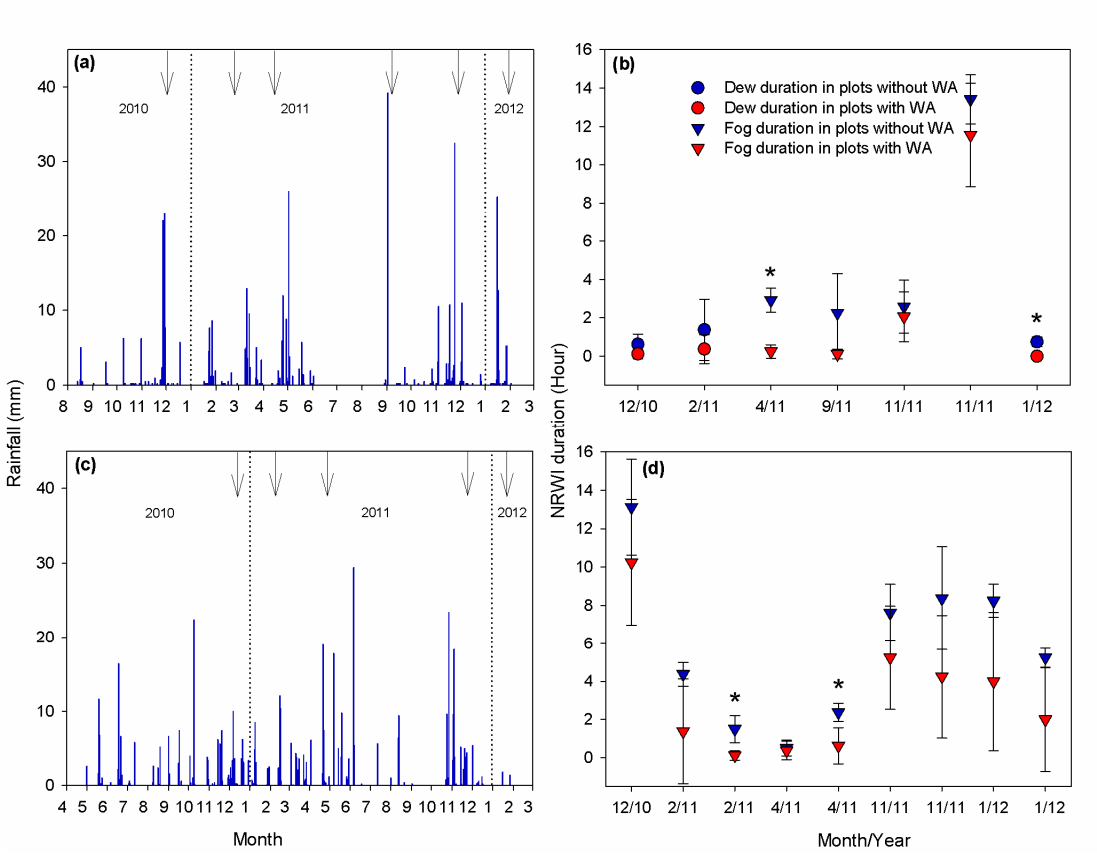

Figure 1 Rainfall registered during the study period at Sorbas **(a)** and Aranjuez **(c)**, and non-rainfall water inputs (NRWI) registered before the gas exchange and chlorophyll fluorescence measurements at Sorbas **(b)** and Aranjuez **(d)**. Asterisks denote statitically significant reduction of NRWI at $p < 0.05$ in warming treatment (WA) plots. NRWI data are means ± SE (n = 4).

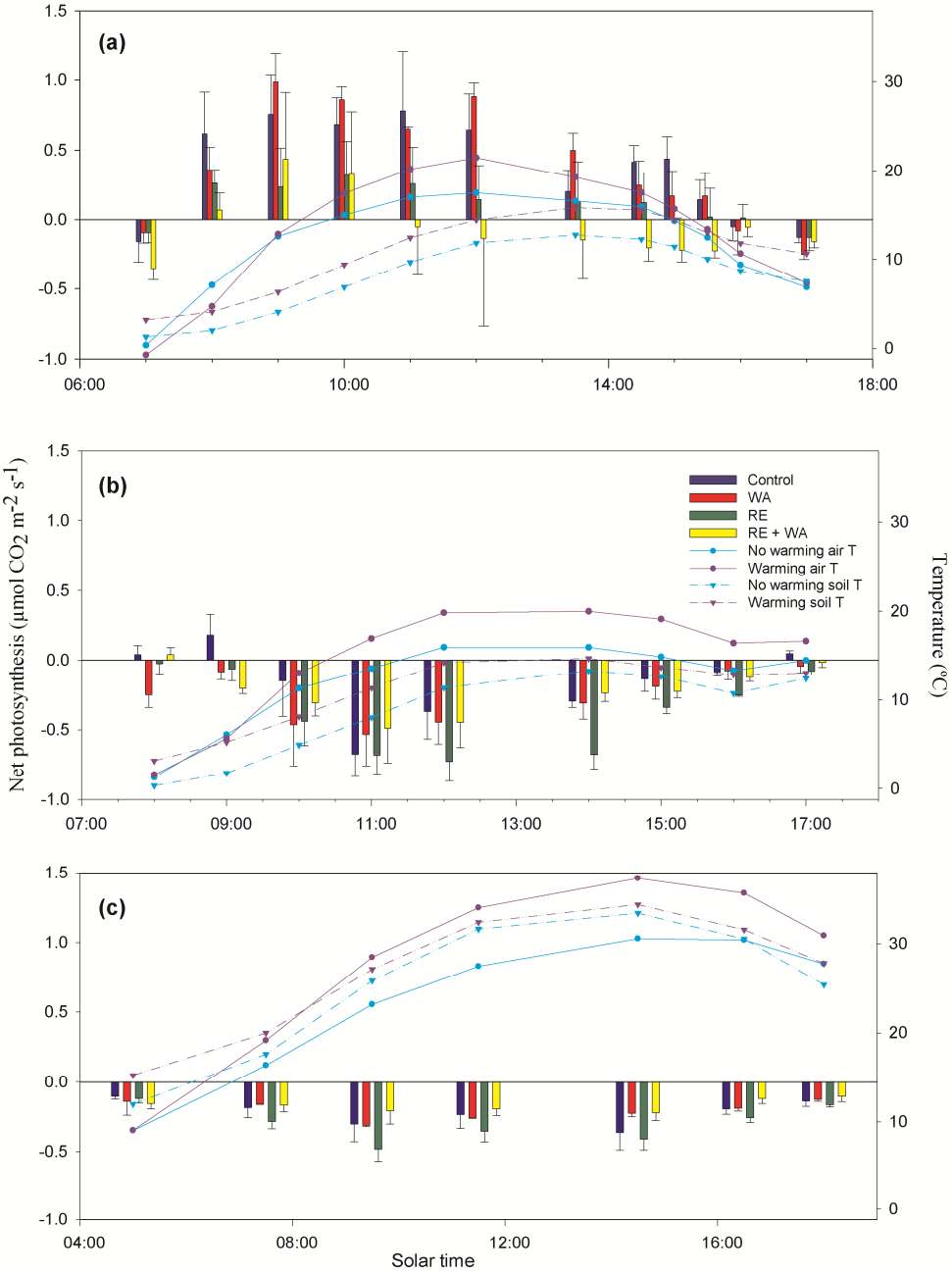

Figure 2 Daily cycle surveys of net photosynthetic rate in January at Sorbas **(a)** and Aranjuez **(b)**, and in May at Aranjuez **(c)**. Data are means ± SE (n = 6 in January; n = 4 in May). WA = warming, and RE = rainfall exclusion.

3.2. Temporal features of the biocrust gas exchange on seasonal and daily scales

Positive net photosynthesis was only detected during late autumn and winter in both Aranjuez and Sorbas (Fig. 3), coinciding with the peak of gross photosynthesis (Fig. 4). The maximum peaks of gross photosynthesis were observed in the high biocrust cover plots at both study sites (Fig. 4). In Sorbas, dark respiration peaks occurred in late

spring in low biocrust cover plots under the RE, WA and RE + WA treatments, and in summer in the rest of the treatments. In Aranjuez, these peaks were detected in spring in low biocrust cover plots under the control, RE and WA treatments, and in the rest of the treatments during late autumn and winter.

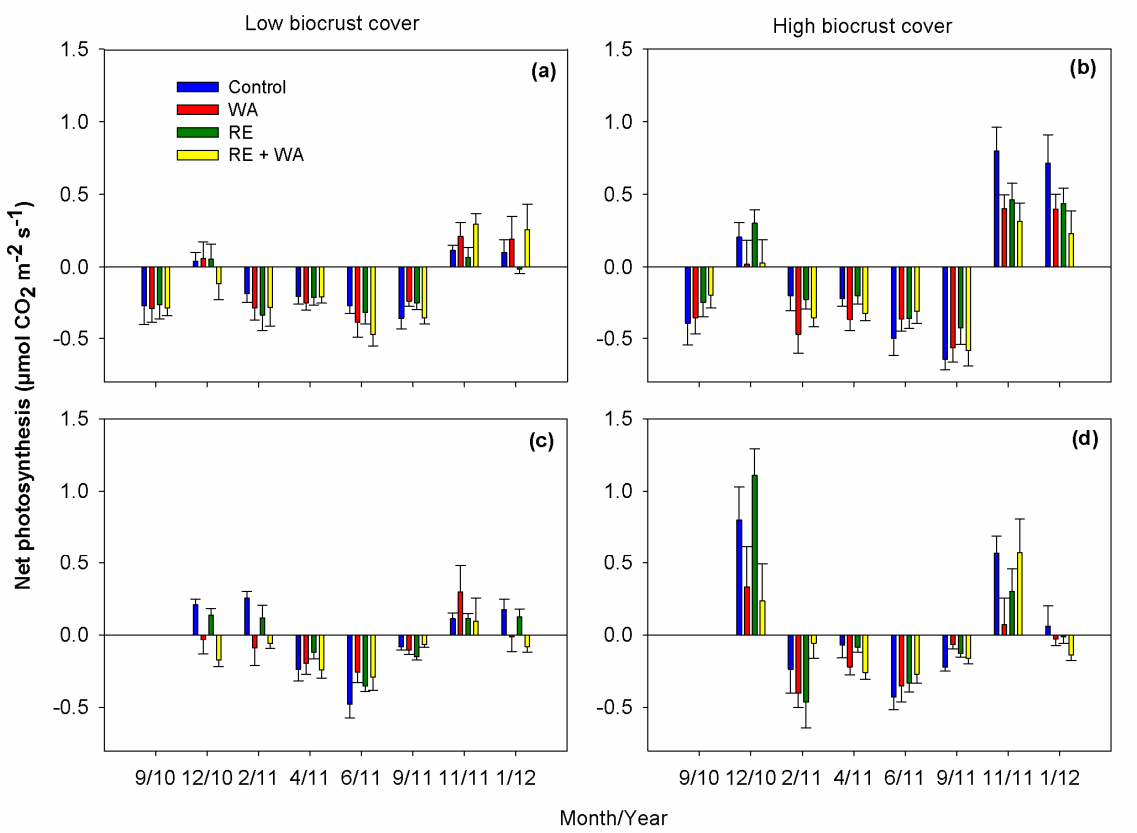

Figure 3 Temporal variation of net photosynthetic rate at Sorbas **(a**, **b)** and Aranjuez **(c**, **d)** experimental sites. Data are means ± SE (n = 6). WA = warming, and RE = rainfall exclusion.

Seasonal effects on net photosynthesis were evident in Aranjuez. Antecedent rainfall inputs between December 2010 and April 2011 were similar, and the differences in other environmental variables (e.g., air and soil temperatures, soil moisture) were not relevant during early morning hours at this site (Figs. S1c, d and S2b, d). The most relevant difference between these two months was the NRWI, which was greater in December (Fig. 1), but presumably its effect on photosynthesis was not significant, since precedent rainfalls were abundant. However, important contrasts in the response of biocrusts were observed, with maximum annual net photosynthesis values in December, and negative values for all treatments in April (Fig. 3c, d); so the biocrust gas exchange response to similar environmental conditions was different depending on the season.

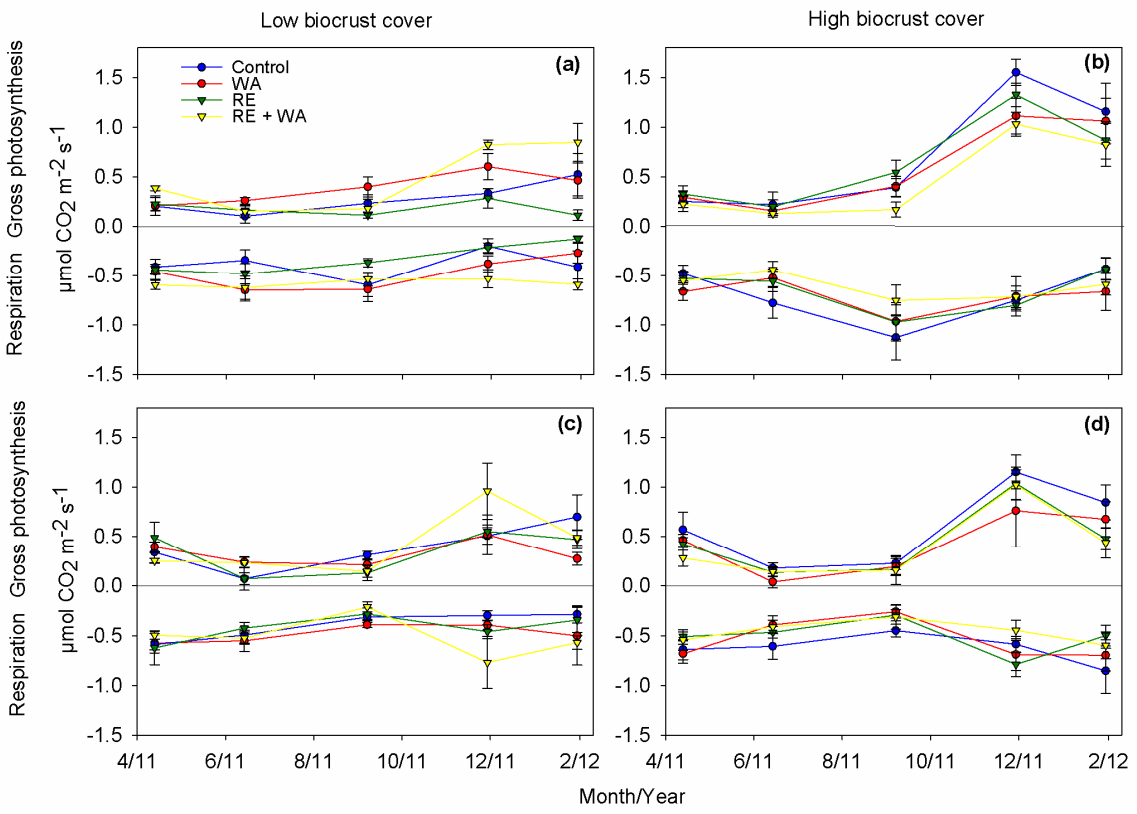

Figure 4 Temporal variation of gross photosynthetic rate and dark respiration at Sorbas **(a**, **b)** and Aranjuez **(c**, **d)** experimental sites. Data are means ± SE (n = 6). WA = warming, and RE = rainfall exclusion.

Due to the random nature of rain events in drylands, the photosynthetic activity can also show a high intra-seasonal variability. For example, the effect of the previous winter rainfalls on *in situ* net photosynthesis over biocrust-forming lichens can be observed comparing December 2010 with February 2011. The carbon fixation differences between these months were remarkable in both sites because preceding precipitation was much more important in the fist than in the second month (Fig. 1).

The January daily cycles obtained at both sites were very different due to the distinct water availability of the specific measured days (Fig. 1, see antecedent rainfalls). This caused differences in gross photosynthesis; as this variable was around three-fold higher in Sorbas than in Aranjuez (Fig. 5). At Sorbas (Fig. 2a), net photosynthesis remained positive in all plots (excepting those under the RE + WA treatment) when PAR values were higher than 150 µmol m^{-2} s^{-1}, and a maximum peak was detected at noon. In Aranjuez (Fig. 2b), positive net photosynthesis was only recorded at dawn and dusk in two treatments (control and RE + WA). During the May

daily cycle in Aranjuez (Fig. 2c), no positive values of net photosynthesis were observed at any time.

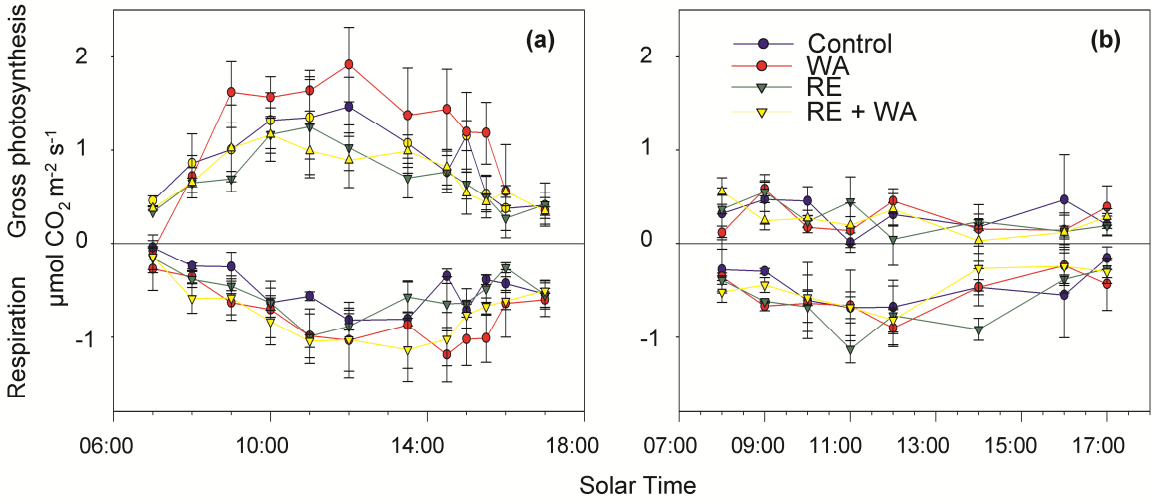

Figure 5 Daily cycle surveys of gross photosynthetic rate and dark respiration in January at Sorbas **(a)** and Aranjuez **(b)** experimental sites. Data are means ± SE (n = 6). WA = warming, and RE = rainfall exclusion.

3.3. Impacts of warming and rainfall reduction on the in situ photosynthetic efficiency and gas exchange of the biocrusts

3.3.1. Variation of the effects of climate change as a function of biocrust cover

In the analysis of the bimonthly surveys, where the factor Crust was included, rainfall exclusion and temperature increase showed complex interactions with biocrust cover and the time of measurement, which affected all the variables measured in Sorbas, and the net photosynthesis in Aranjuez. Warming had a greater effect on biocrust performance at Sorbas than at Aranjuez, and reduced carbon fixation in areas with well-developed biocrusts. Significant interactions found with the statistical models are disclosed below in detail.

A significant Date × Crust × WA interaction was found when analyzing F_v/F_m data from Sorbas (Table Supplementary material (S) 1). An analysis of the Crust × WA interaction for the different surveys revealed that, in December 2010, plots with low biocrust cover and without OTCs had significantly lower F_v/F_m values than those with high biocrust cover plots (regardless of the presence of OTCs). In November 2011, low

biocrust cover plots had significantly lower F_v/F_m values than high biocrust cover plots, regardless of the WA levels considered. No significant differences were found in the factor RE (Fig. 6d).

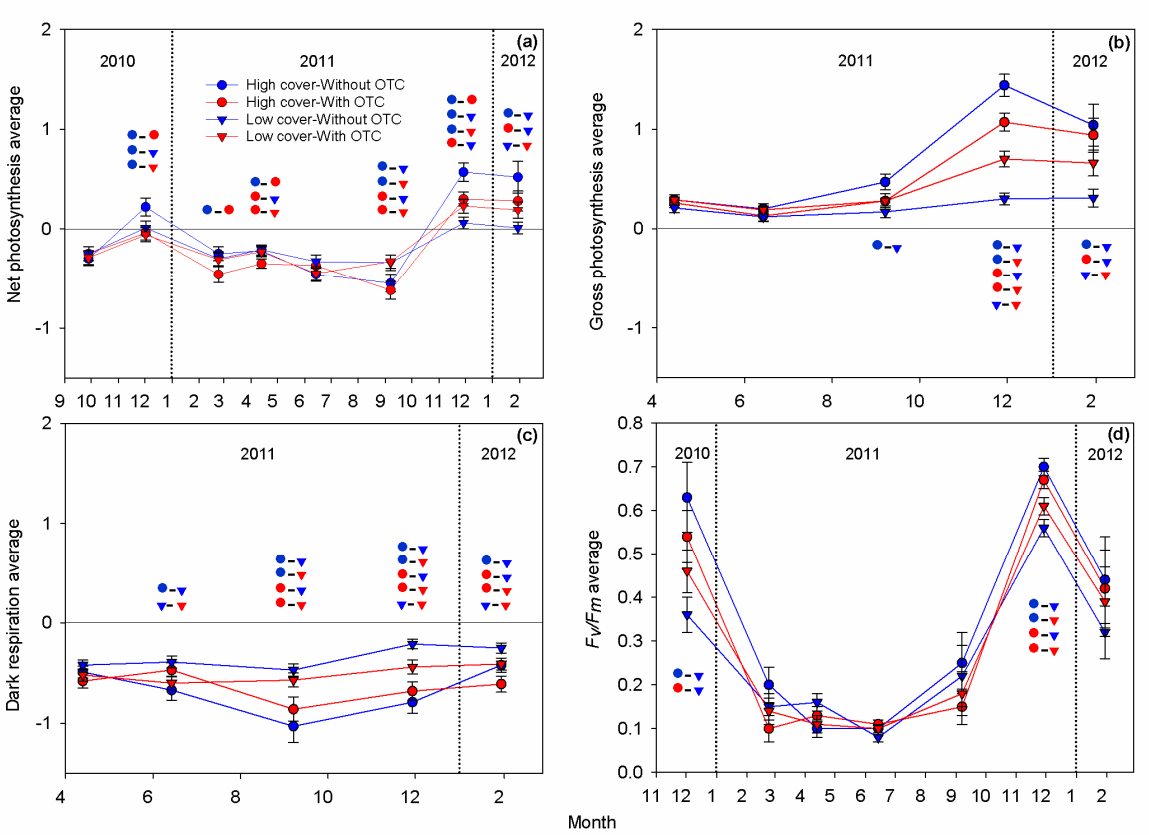

Figure 6 Fisher's least significant difference (LSD) post-hoc results in Sorbas bimonthly surveys: net photosynthetic rate **(a)**, gross photosynthetic rate **(b)**, dark respiration **(c)** and F_v/F_m **(d)**. Treatment symbols joined by dashes denote statistically different averages at $p < 0.05$ between these treatments within each time. Data are means ± SE (n = 6). WA = Warming.

When analyzing net photosynthesis data, a significant Date × Crust × WA interaction was also found at Sorbas (Table S2). When this variable was positive, WA reduced it in high biocrust cover plots (Fig. 6a). But in low biocrust cover plots, net photosynthesis was increased with WA during the last measured winter month. High biocrust cover plots had greater positive carbon balance than low biocrust cover plots without OTCs, but these differences were not found in the WA treatments. In February and April 2011, when net photosynthesis was negative, WA increased the carbon emissions in high biocrust cover plots, an effect that was not found in the low biocrust cover plots. No significant effects of RE were found in Sorbas, but in Aranjuez a significant Date × Crust × WA × RE interaction was found (Table S2). In December

2010, control and RE plots with high biocrust cover had a significantly higher net photosynthesis than those from other treatments (Fig. 7a). However, only low biocrust cover plots with control and RE treatments had a positive net photosynthesis in February 2011 (Fig. 7b). At this date, the values found in these plots were significantly greater from those found in high biocrust cover plots with either WA or RE, but not with WA + RE treatments.

A significant Date × Crust × WA interaction was found when analyzing gross photosynthesis data from Sorbas (Table S2). Only low biocrust cover plots had significant differences between WA factor levels, as this variable was higher in WA plots during late autumn and winter. In plots without OTCs, gross photosynthesis was significantly higher in high biocrust cover plots than in low biocrust cover plots during September 2011, November 2011 and January 2012 (Fig. 6b). In WA plots, this difference was only found in November 2011. In Aranjuez, a significant Date × Crust interaction was found; high biocrust cover plots had significantly higher values of gross photosynthesis than low biocrust cover plots in late autumn, when maximum photosynthetic rates were reached (Fig. 7c). Rainfall exclusion had not significant effects on gross photosynthesis.

A Date × Crust × WA interaction close to statistical significance was found when analyzing dark respiration in Sorbas (Table S2). As when F values exceed 2 there is a high probability that the results would have been significant with more replicates (Fry et al., 2013), a post-hoc analysis for this interaction was performed. Only low biocrust cover plots showed significant differences between WA levels; higher dark respiration was observed in WA plots in late spring (Fig. 6c). Differences between Crust factor levels were also observed: in plots without OTC high biocrust cover showed higher dark respiration than low biocrust cover in four months (June 2011, September 2011, November 2011 and January 2012); this was also found in plots with OTC in September 2011 and January 2012. In Aranjuez, Date significantly affected dark respiration, with the highest monthly average found in April, (Fig. 7d).

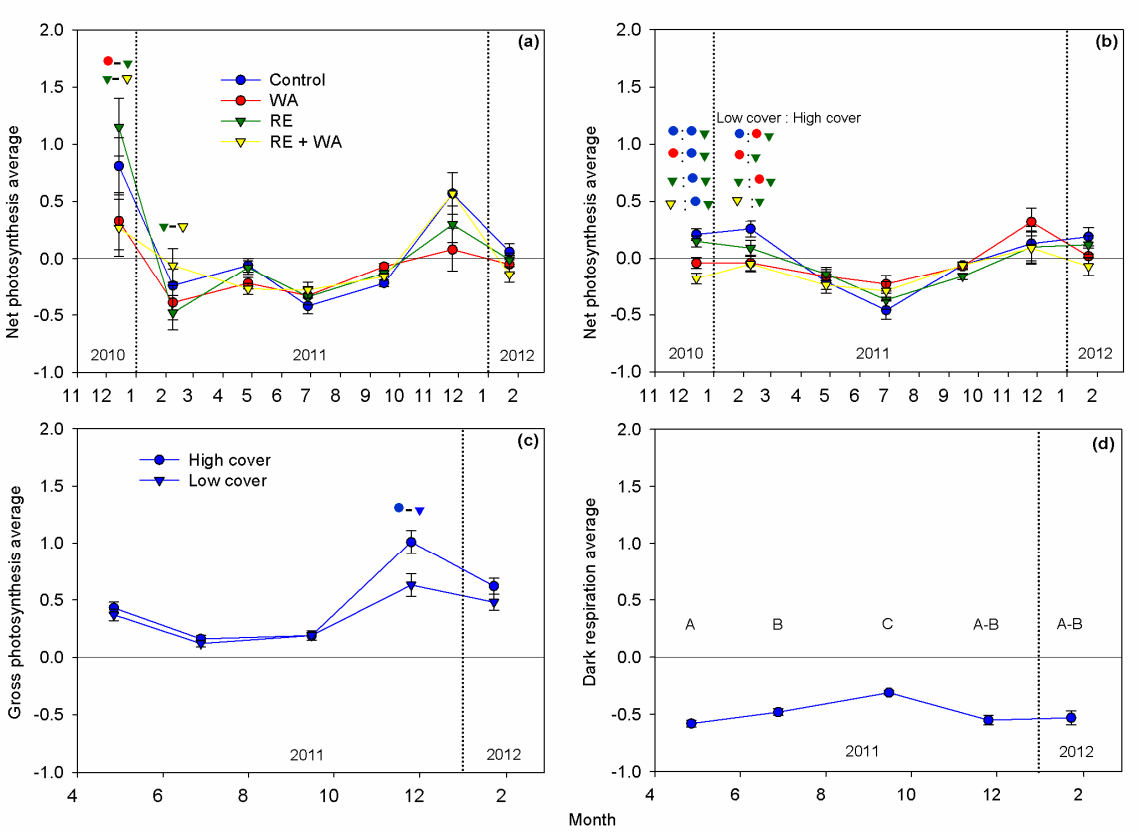

Figure 7 Fisher's least significant difference (LSD) post-hoc results in Aranjuez bimonthly surveys: net photosynthetic rate in high biocrust cover plots **(a)**, net photosynthetic rate in low biocrust cover plots **(b)**, gross photosynthetic rate **(c)** and dark respiration **(d)**. In panels **(a)** and **(c),** the pairs of treatments with statistically different averages within each time are denoted by their legend symbols joined by dashes. In panel **(b)**, there are no significant differences between the treatments represented within each time. However, significant differences between biocrust cover treatments (i.e. statistical comparison between panels **(a)** and **(b)**) within each time are shown above the averages. The first column of symbols represents the low cover treatments, and after colons, the high cover treatments where significant differences were found. In panel **(d)**, significant differences between surveys are shown by letters. No letters in common denote different averages. All analyses were performed at $p < 0.05$. Data are means ± SE (n = 6).WA = warming, and RE = rainfall exclusion.

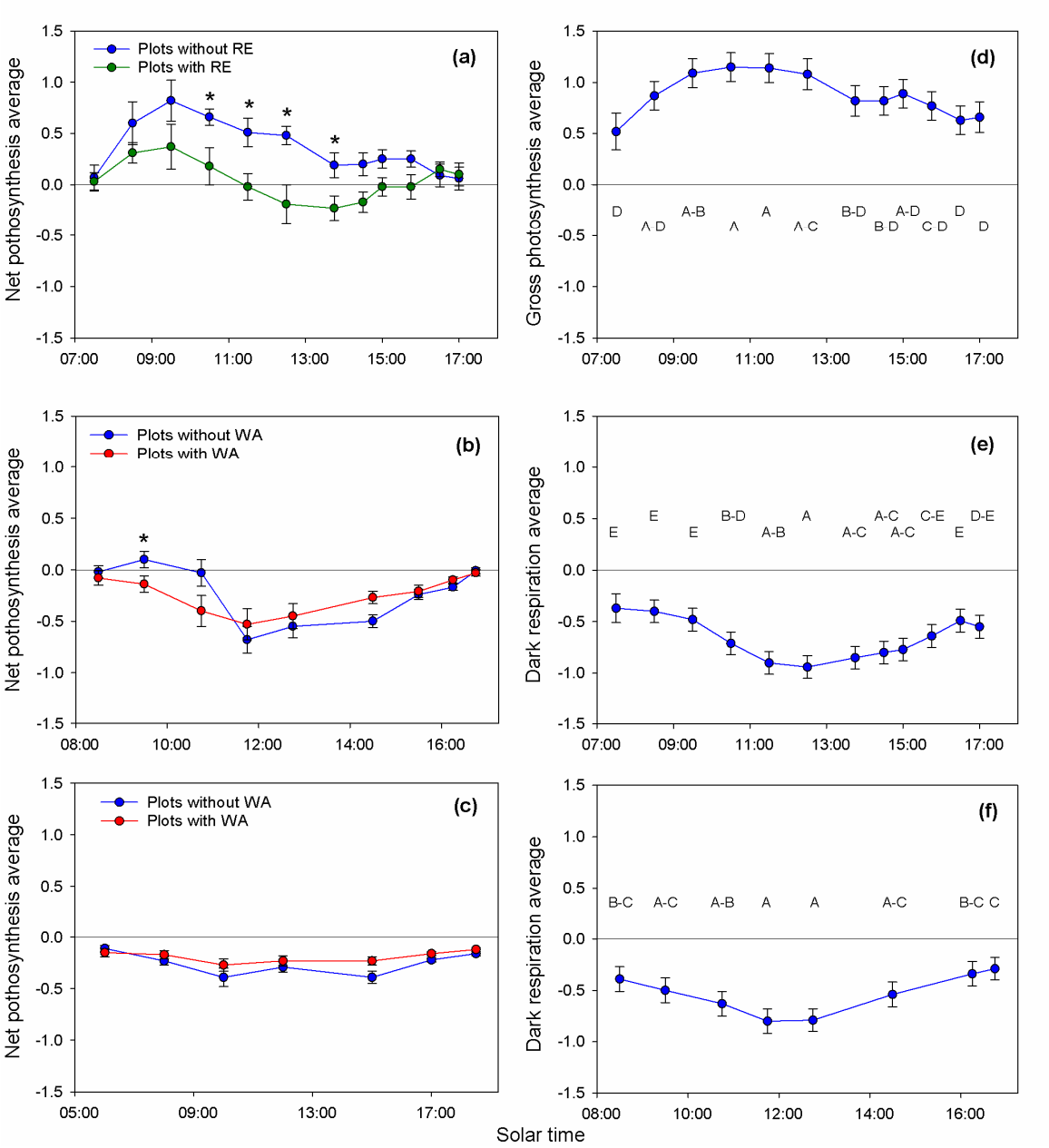

Figure 8 Fisher's least significant difference (LSD) post-hoc results from daily cycle surveys: net photosynthetic rate **(a)**, gross photosynthetic rate **(d)** and dark respiration **(e)** in January at Sorbas**;** net photosynthetic rate **(b)** and dark respiration **(f)** in January at Aranjuez; and net photosynthetic rate **(c)** in May at Aranjuez. In panels **(a)**, **(b)** and **(c),** statistical differences between the two treatments within each time are denoted by asterisks. In panels **(d)**, **(e)** and **(f)**, significant differences between surveys are shown by letter intervals (e.g., a label A-C means that a time contains the letters A, B and C). No letters in common denote different averages. All analyses were performed at $p < 0.05$. Data are means ± SE (n = 6). WA = warming, and RE = rainfall exclusion.

3.3.2. Variation of the effects of climate change as a function of the time scale and water availability

The effects of our treatments also differed with the time scale considered (bimonthly or daily measurements), and with the characteristics of the preceding rainfall and NRWI events. For example, although warming was the most important factor determining responses in Sorbas at early morning hours during bimonthly measurements, rainfall exclusion was the main factor limiting net photosynthesis during the winter daily cycle at this site, as a significant Time × RE interaction was found (Table S3). Plots with rainfall exclusion had significantly lower net photosynthesis than plots without it from 10 A.M to 2 P.M. (Fig. 8a). So natural precipitation maintained soil moisture at suitable levels allowing photosynthetic activity throughout the day, something that was not achieved when rainfall was experimentally reduced.

In Aranjuez, where rainfalls preceding the winter daily cycle measurements were much lower than in Sorbas (Fig. 1), a significant Time × WA interaction was found in both January and May (Tables S3 and S4). In January, net assimilation rate was higher and positive in plots without OTC, and negative in plots with OTC, but only from 9 A.M. to 10 A.M (Fig. 8b). In May, the post-hoc test did not show significant differences between plots with and without OTC measured in the same hour (Fig. 8c).

Regarding the other gas exchange variables, significantly higher gross photosynthesis values were found in the morning than in the afternoon in Sorbas (Fig. 8d), while none of the factors evaluated affected gross photosynthesis in Aranjuez (Table S3). The dark respiration values found were similar in Aranjuez and Sorbas, and at both sites peaked at midday (Fig. 8e, f, Table S3).

4. Discussion

4.1. The importance of water availability on biocrust activity

In agreement with previous studies (Lange & Green, 2003; Wilske et al., 2008), our results show that seasonal variations in the physiological status and the CO_2 exchange of biocrust-dominated microsites are strongly influenced by the hydration state of biocrust constituents. The maximum photochemical efficiency of PSII (F_v/F_m) reflected the typical physiological activity of biocrusts in the Mediterranean, characterized by a

strong seasonal oscillation, with a peak of activity in winter, the rainy season of this climate, and a phase of dormancy in summer, the dry season (Wilske et al., 2008; Pintado et al., 2010). The drastic reduction of F_v/F_m observed during spring and summer (Fig. 6d) indicates a photoinhibition situation resulting from damage to PSII reaction centers, or a down-regulation of PSII (Calatayud et al., 1997). We favour the predominance of the second explanation, as when biocrusts returned to better moisture conditions they recovered the maximum F_v/F_m values, and had similar photosynthetic rates to those observed before the dry season. However, biocrust did not reach an optimal photochemical efficiency at our study sites until October, as was shown by Pintado et al. (2010), who monitored *in situ* chlorophyll fluorescence of biocrust-forming lichens during several years in a site near to Sorbas. This can be seen with the September 2011 measurements in Sorbas, which were conducted after a relevant rain, and with soil temperature values suitable for the activity of biocrusts. In spite of these conditions, respiration was greater than photosynthesis, generating the most negative carbon balance (carbon source) found at this site.

Hydration status also had an important role in the metabolic activity of biocrusts over the day. The differences observed in the January daily cycle between Aranjuez and Sorbas can be explained by the unequal rainfall conditions among them. The major water inputs, and consequently higher soil water content found in Sorbas during this month, allowed optimal conditions for carbon fixation along the day (Fig. 2a), whereas in Aranjuez the lower rainfalls only allowed positive carbon fixation at early and late hours (Fig. 2b). In May 2010, and while ten days prior to the measurements a rain event of ~ 10 mm took place in Aranjuez, the net photosynthesis was negative throughout the day (Fig. 2c), probably because an important part of the water supply had already evaporated, and also because at that time of the year the photosynthetic capacity of lichens is lower than in winter.

4.2. Water pulses and degree of biocrust development as modulators of its responses to climate change

The occurrence, magnitude and timing of the two NRWI sources considered (dew and fog) are key to interpret the results and the differences found between Sorbas and Aranjuez during the bimonthly surveys. The RE treatment reduced the amount of rainfall reaching the plots, but did not affect the water inputs coming from NRWI.

Nocturnal and early morning moistening through dew and fog enables photosynthesis immediately after sunrise, which ceases when biocrusts dry out (Kappen, 1988), after ca. 2 h. However, high air temperature accelerates the process of thallus dehydratation, a major environmental stressor in lichens (Hájek et al., 2006). Given all this, the main effects of the warming treatment in our experiment were: (i) the reduction of the early morning wetting time on the thalli, by decreasing the formation of dew on the surface of the biocrusts and intercepting fog; and (ii) the reduction of the soil water content because the warming increased evaporation. Therefore the negative effect of WA treatment on biocrust photosynthetic performance was due to its reduction of water availability, also in agreement with Maphangwa et al. (2012). Direct effects of warming on the photosynthetic efficiency of biocrusts can be discarded, as the early morning temperature ranges in plots with and without OTC (Fig. S2) were within the photosynthetic optimal of the dominant lichens from our study sites (Lange et al., 1997, 1998; Lange & Green, 2004). Johnson et al. (2012) and Zelikova et al. (2012) also found very limited effects of a 2–3ºC warming alone on biocrust communities dominated by cyanobacteria, cyanolichens and mosses.

Warming had a greater effect on gas exchange variables in Sorbas than in Aranjuez in the bimonthly survey, and this was likely due to the differences on NRWI magnitude observed among the study sites. In Sorbas, dew is the most frequent water input. For example, in a study conducted near this site, dewfall condensation and rain/fog inputs were observed during 78% and 16% of the nights, respectively (Uclés et al., 2013a). However, fog is the main NRWI source in Aranjuez, as it occured during 27% of the nights of this study period, while rainfalls only occured during 8% of the nights. In all measured winter months, there was dew formation at Sorbas, and fog events were encountered at Aranjuez (Fig. 1). Since dew is directly related with the temperature of the soil surface, warming reduced dew condensation. Our OTCs were also a barrier to the entry of the fog into the plots, but as they were open at the top and elevated from the soil, only reduced significantly the duration of the small fog events. This effect on small NRWI events probably is critical for early net carbon balance, since they may be at the limit of providing enough water to compensate and surpass respiration activity. So, as warming had a greater effect on small NRWI events, which were more frequent in Sorbas, this could be the main explanation of why the effects of WA factor were most significant in Sorbas than in Aranjuez when analyzing bimonthly data.

Contrary to what we expected, rainfall exclusion did not have a detrimental effect at Sorbas in the bimonthly surveys, and even this treatment had higher net photosynthesis than in plots with WA and WA + RE treatments (Fig. 7a) at Aranjuez in December 2010. This fact may also be explained because of the importance of the antecedent NWRIs. During the nighttime previous to the measurements, there were 13 hours of fog at Aranjuez, and the rainfall shelters did not reduce the amount of fog as the OTCs did (Fig. 1). Therefore, these results could indicate a most important modulation of the early photosynthesis by NRWI pulses than by rainfalls along an annual cycle. When preceding precipitation was abundant, rainfall exclusion had an effect on the evolution of gas exchange during the whole day, as in the case of Sorbas (Fig. 8a). However, when rainfalls were not large enough to maintain a positive carbon balance during the whole day, as ocurred in the Aranjuez winter daily cycle, temperature became the main factor driving biocrust activity (Fig. 8b). The observed net photosynthesis in control plots during early morning at this site was probably activated by nocturnal fog events, and the WA treatment, that caused an increase of soil temperature of about 3–4ºC (Fig. 2b), increased the dehydration rate of the thalli, and impeded a positive carbon balance in this treatment when light conditions were more favorable.

Although the data acquisition of our work was not extensive enough to quantify the relative importance of rain and NRWI in the biocrusts gas exchange, their variability, frequency and timing characteristics suggest that reducing NRWI pulses suitable for carbon fixation could have a greater impact on the biocrust carbon balance at annual scale. For example, other studies performed in the desert of Neveg consider that dew is a more important source of water for biological activity than rain because (i) its lower inter-annual variability, 12%, compared to 53% for rainfall (Zangvil, 1996); (ii) its more frequency, 200 days of dew vs. 20 days of rain (Temina & Kidron, 2011); and (iii) its well distribution over the year, a condition necessary for the formation of biological crust, as this implies that some moisture must be available on a regular basis (Jacobs et al., 1999). The important role of NRWIs in these dryland organisms have also been supported by several studies (e.g., Lange et al., 1994; del Prado & Sancho, 2007; Kidron & Temina, 2013).

During late autumn and winter, the F_v/F_m values observed in low biocrust cover plots were similar to those observed in high cover plots (Fig. 6d), indicating that the "bare" soil without a well developed and visible biocrust community possessed an

abundant colony of free-living cyanobacteria and algae. This was consistent with our gross photosynthetic data (Fig. 6b). Cyanobacteria and algae constitute the early-successional stage during the development of biocrust communities (Garcia-Pichel et al., 2001; Lázaro et al., 2008), and have been described in worldwide dryland regions (Garcia-Pichel et al., 2003; Maestre et al., 2011). The positive effect of warming on the gross photosynthesis of low biocrust cover plots at Sorbas could have been produced because, when hydrated enough, free and lichenized cyanobacteria have a much stronger photosynthesis increase response to temperature than lichen species with green algae as photobiont (Lange et al. 1998; Housman et al. 2006). Dark respiration in low biocrust cover plots at Sorbas was significantly higher with warming in June (Fig. 6c), while this treatment did not affect dark respiration at high cover plots on any season. The respiration response to WA in free cyanobacteria, and the absence of it in green-algal lichens may indicate that the respiration of the first also had a greater sensitivity to temperature increase, or that the latter exhibited higher acclimatization capacity, as their fungal component possesses this ability (Lange & Green, 2005).

Cyanobacteria possess a more efficient CO_2 concentrating mechanism (CCM) than green algae (Green et al., 1993; Badger et al,. 2006; Price et al., 2008), which counteracts the increase of Rubisco oxygenase activity with temperature, leading to a photorespiration rise (Palmqvist et al., 1994; Smith & Griffiths, 1998). Therefore, the CCM mechanism may be more beneficial at high temperatures, where this energy cost is smaller than the energy associated with photorespiration. This difference may also explain why cyanobacterial crusts are often the only biocrust type in arid and hyperarid ecosystems, and their gross photosynthesis has a resilience to change at temperatures between 20 and 35ºC (Grote et al., 2010). It is also possible that under conditions of less NRWI formation, cyanobacteria have a competitive advantage to avoid dessication over green algae lichens in terms of water-holding capacity (Lange et al., 1998), production of a protective layer of exopolysaccharides (De Philippis & Vincenzini, 1998; Potts, 2001), or even migration abilities through soil profile (Garcia-Pichel & Pringault, 2001). These characteristics of cyanobacteria could explain why the drying effect of WA did not cause a negative response on the net photosynthesis of low biocrust cover plots (Fig. 6a).

5. Concluding remarks

Our results illustrate how the timing and magnitude of water pulses drive key ecological processes in dryland ecosystems, in this case, the carbon cycle linked to the metabolic activity of biocrusts. The shifts in these pulses caused by climate change will determine the magnitude of the effects on this biological activity, which will determine responses to warming of the whole carbon cycle (Maestre et al., 2013). Our findings also emphasize the importance of NRWIs when understanding biocrust responses to climate change. They indicate that changes in NRWI regimes as consequence of warming could have a greater impact on the carbon balance of biocrusts than changes in rainfall amounts. Another contribution of this study is the differential response of the biocrusts to the climate change treatments as a function of their development stage. The results suggest that early-successional cyanobacterial biocrust could displace more developed lichen-dominated biocrusts in Mediterranean drylands, as indeed is happening in Aranjuez, the experimental site with more years of climate manipulation (Escolar et al., 2012). These changes induced by climate change have implications for the carbon cycle in the studied ecosystems, as early-successional biocrust areas have less carbon fix capacity. Consequently, the importance of dryland ecosystems as a carbon sink will be reduced if these shifts in the biocrust community take place.

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SUPPLEMENTARY MATERIAL

Simulated climate change reduced the capacity of lichen-dominated biocrusts to act as carbon sinks in two semi-arid Mediterranean ecosystems

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Table S1 Results of the MIXED model analysis evaluating the treatments effects on the F_v/F_m of biocrusts in Sorbas. Date = date on which measurement took place; Crust = biocrust cover (< 25% vs. > 75%); WA = warming; RE = rainfall exclusion. P values below 0.05 are in bold; those between 0.05 and 0.10 are in italics.

F_v/F_m Bimonthly Survey	DF	F-value	p-value
Date	6, 117	442.70	**<0.001**
Crust	1, 42	6.86	**0.008**
WA	1, 42	0.32	0.568
RE	1, 42	1.09	0.300
Date × Crust	6, 117	6.71	**<0.001**
Date × WA	6, 117	1.12	0.347
Date × RE	6, 117	0.20	0.974
Crust × WA	1, 42	2.83	*0.070*
Crust × RE	1, 42	0.79	0.469
WA × RE	1, 42	0.01	0.920
Date × Crust × WA	6, 117	2.76	**0.011**
Date × Crust × RE	6, 117	0.98	0.546
Date × WA × RE	6, 117	0.59	0.736
Crust × WA × RE	1, 42	0.05	0.948
Date × Crust × WA × RE	6, 117	0.39	0.716

Table S2 Results of the MIXED model analysis evaluating the treatments effects on gas exchange of biocrusts at Sorbas and Aranjuez study sites. Rest of legend as in Table S1.

Bimonthly Survey	**SORBAS**								
	Net photosynthesis			Gross photosynthesis			Dark respiration		
	DF	F-value	p-value	DF	F-value	p-value	DF	F-value	p-value
Date	7, 269	68.46	**<0.001**	4, 150	61.13	**<0.001**	4, 150	6.74	**<0.001**
Crust	1, 49	1.57	0.216	1, 49	0.20	0.660	1, 49	1.25	0.277
WA	1, 49	3.93	*0.053*	1, 49	0.33	0.578	1, 49	2.48	0.127
RE	1, 49	0.14	0.708	1, 49	1.26	0.272	1, 49	0.21	0.652
Date × Crust	7, 269	6.98	**<0.001**	4, 150	17.68	**<0.001**	4, 150	5.04	**<0.001**
Date × WA	7, 269	0.81	0.581	4, 150	0.30	0.889	4, 150	1.21	0.314
Date × RE	7, 269	0.73	0.651	4, 150	1.15	0.348	4, 150	1.03	0.402
Crust × WA	1, 49	5.88	**0.019**	1, 49	1.57	0.221	1, 49	0.00	0.974
Crust × RE	1, 49	0.06	0.804	1, 49	1.22	0.271	1, 49	1.12	0.306
WA × RE	1, 49	0.07	0.792	1, 49	0.00	0.965	1, 49	0.12	0.739
Date × Crust × WA	7, 269	2.90	**0.006**	4, 150	3.37	**0.012**	4, 150	2.19	*0.071*
Date × Crust × RE	7, 269	1.31	0.247	4, 150	0.96	0.438	4, 150	0.30	0.871
Date × WA × RE	7, 269	1.11	0.360	4, 150	1.90	0.110	4, 150	1.32	0.279
Crust × WA × RE	1, 49	0.08	0.784	1, 49	2.87	0.104	1, 49	1.55	0.229
Date × Crust × WA × RE	7, 269	0.12	0.997	4, 150	1.18	0.322	4, 150	1.25	0.292
	ARANJUEZ								
Date	6, 216	32.93	**<0.001**	4, 135	45.20	**<0.001**	4, 135	15.30	**<0.001**
Crust	1, 216	1.96	0.163	1, 135	0.83	0.363	1, 135	0.15	0.700
WA	1, 216	6.78	**0.009**	1, 135	1.82	0.180	1, 135	0.03	0.872
RE	1, 216	0.15	0.706	1, 135	1.61	0.2106	1, 135	1.64	0.202
Date × Crust	6, 216	10.10	**<0.001**	4, 135	3.18	**0.016**	4, 135	1.67	0.159
Date × WA	6, 216	7.58	**<0.001**	4, 135	1.26	0.299	4, 135	0.37	0.827
Date × RE	6, 216	0.53	0.788	4, 135	1.23	0.299	4, 135	1.05	0.386
Crust × WA	1, 216	0.64	0.424	1, 135	0.16	0.686	1, 135	0.24	0.622
Crust × RE	1, 216	2.00	0.168	1, 135	1.11	0.292	1, 135	0.59	0.443
WA × RE	1, 216	0.14	0.708	1, 135	1.41	0.237	1, 135	0.28	0.595
Date × Crust × WA	6, 216	2.42	**0.027**	4, 135	1.20	0.313	4, 135	0.64	0.632
Date × Crust × RE	6, 216	0.48	0.826	4, 135	1.18	0.323	4, 135	1.32	0.266
Date × WA × RE	6, 216	1.76	0.109	4, 135	1.55	0.191	4, 135	0.92	0.454
Crust × WA × RE	1, 216	1.09	0.298	1, 135	0.72	0.398	1, 135	0.16	0.685
Date × Crust × WA × RE	6, 216	3.50	**0.003**	4, 135	0.80	0.527	4, 135	1.94	0.108

Table S3 Results of the MIXED model analysis of the climate change factors effects on biocrusts gas exchange in January daily cycle surveys in Sorbas and Aranjuez. Time = hour of the day at which measurement took place; PAR = covariate Photosynthetic Active Radiation (µmol photon m^{-2} s^{-1}). Rest of legend as in Table S1.

Daily cycle survey in January	SORBAS								
	Net photosynthesis			Gross photosynthesis			Dark respiration		
	DF	F-value	p-value	DF	F-value	p-value	DF	F-value	p-value
Time	11, 87	4.85	**<0.001**	11, 78	2.00	**0.046**	11, 79	4.73	**<0.001**
WA	1, 6	0.07	0.803	1, 6	1.93	0.213	1, 6	3.19	0.124
RE	1, 6	5.63	*0.062*	1, 6	3.23	0.126	1, 6	0.05	0.835
PAR	1, 87	95.92	**<0.001**	1, 78	16.94	**<0.001**			
Time × WA	11, 87	0.82	0.622	11, 78	1.22	0.298	11, 79	1.07	0.406
Time × RE	11, 87	2.28	**0.029**	11, 78	1.12	0.365	11, 79	0.60	0.827
WA × RE	1, 6	0.35	0.588	1, 6	0.38	0.568	1, 6	0.00	0.953
Time × WA × RE	11, 87	0.66	0.770	11, 78	0.94	0.500	11, 79	1.36	0.217
	ARANJUEZ								
Time	8, 65	23.83	**<0.001**	7, 49	0.79	0.600	7, 49	3.12	**0.008**
WA	1, 9	0.05	0.829	1, 9	0.06	0.819	1, 9	0.07	0.804
RE	1, 9	1.16	0.309	1, 9	0.06	0.812	1, 9	0.17	0.693
Time × WA	8, 65	4.22	**<0.001**	7, 49	0.01	0.934	7, 49	0.84	0.562
Time × RE	8, 65	1.43	0.200	7, 49	0.69	0.683	7, 49	0.26	0.966
WA × RE	1, 9	2.33	0.161	1, 9	0.53	0.812	1, 9	0.94	0.358
Time × WA × RE	8, 65	0.71	0.680	7, 49	0.77	0.612	7, 49	0.46	0.859

Table S4 Results of the MIXED model analysis of the climate change factors effects on biocrusts net photosynthesis in May daily cycle survey in Aranjuez. Rest of legend as in Table S3

Daily cycle survey in May	ARANJUEZ		
	Net photosynthesis		
	DF	F-value	p-value
WA	1, 9	1.72	0.223
RE	1, 9	0.32	0.587
Time	6, 41	11.01	**<0.001**
WA × RE	1, 9	1.06	0.331
WA × Time	6, 41	2.60	**0.032**
RE × Time	6, 41	0.41	0.870
WA × RE × Time	6, 41	1.13	0.362

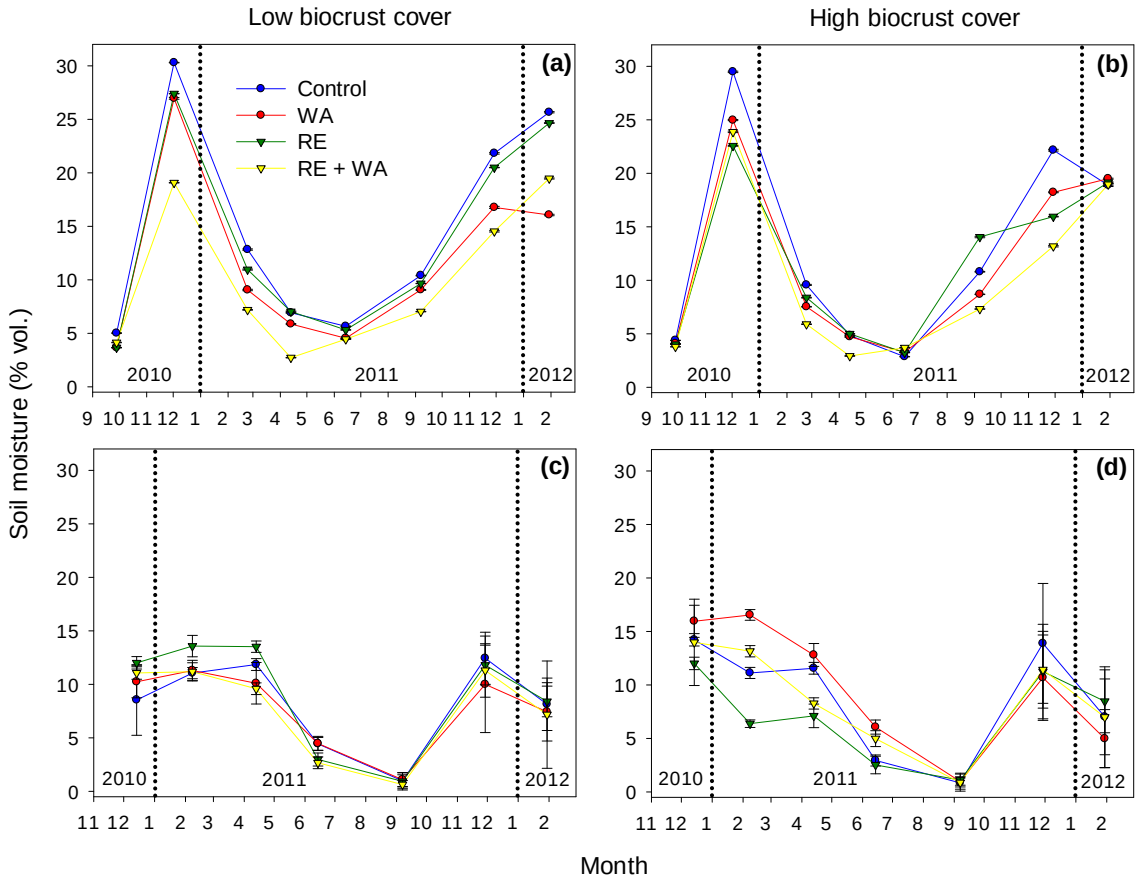

Figure S1 Treatment effects on soil moisture on the gas exchange measurement days at Sorbas **(a**, **b)** and at Aranjuez **(c**, **d)**. Data are means ± SE (n = 3). WA = Warming, and RE = Rainfall exclusion.

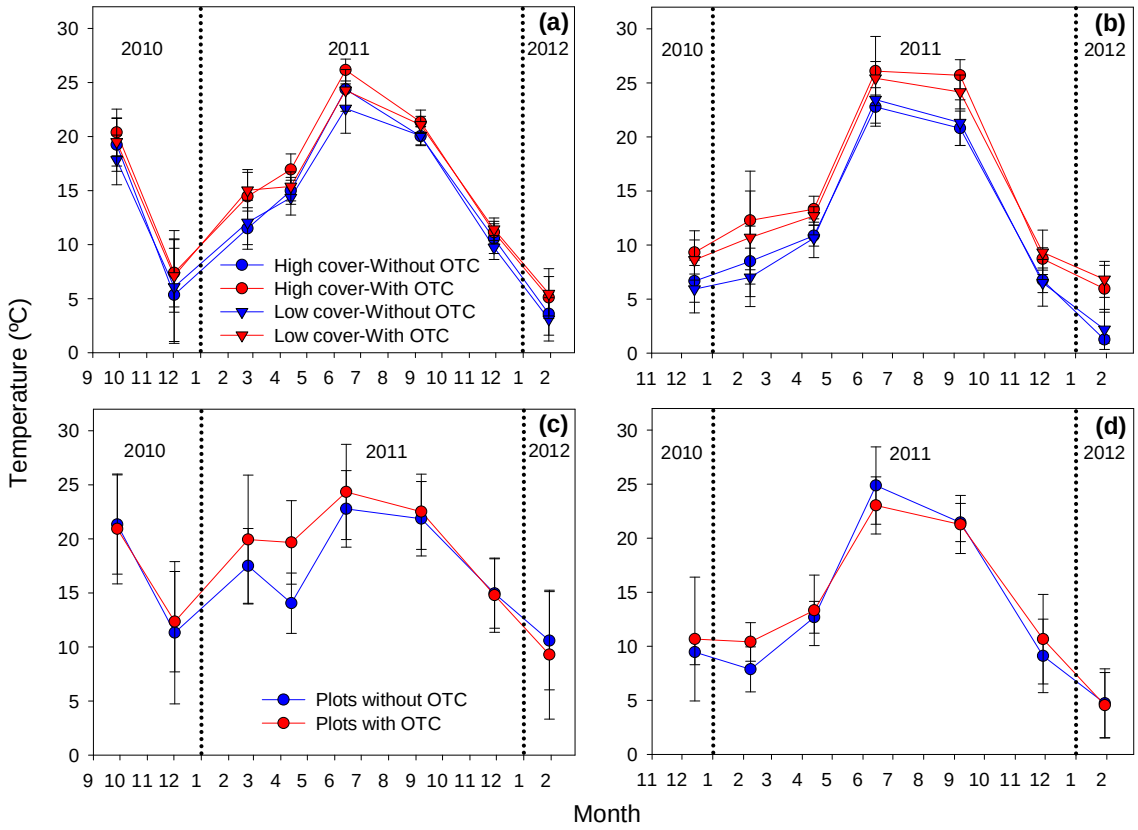

Figure S2 Warming treatment (WA) effects on soil and air temperature at Sorbas **(a**, **c)** respectively; and at Aranjuez **(b**, **d)** respectively. Data are means ± SE (n = 4).

General discussion

GENERAL DISCUSSION

1. Technical methodology developed in this thesis

The implementation of this thesis has required the design and building of two instruments: (i) a custom chamber for measuring *in situ* gas exchange on unaltered biological soil crusts (BSCs) described in Chapter III; and (ii) a mobile rain-out shelter to be able to carry out the desired irrigation schedules with minimal alteration of the natural environment used in Chapters I and II. Here we briefly discuss the antecedents of the applications of these tools with their advantages and disadvantages, as well as the decisions about the final design of our custom devices according to the specific requirements of the experimental designs in this work.

1.1 Portable chamber for in situ CO_2 exchange measurements on BSC

Research into photosynthesis has received considerable stimulus since the 1980s thanks to the availability of accuracy systems based on IRGA technology (Russell & Botha, 1988). One of the first techniques used to measure gas exchange in poikilohydric organisms in field conditions was by means of a porometer chamber for higher plants connected to an IRGA and adapted to measure on foliose and fruticose lichens (Lange et al., 1984). Afterwards, this technique was adapted to measure on epilithic lichens *in situ* or under seminatural conditions, i.e., measurements were performed near the natural site, but the samples were collected (e.g., Kappen et al., 1986; 1990; 1996; Kappen, 1989; Kappen & Breuer, 1991; Sojo et al., 1997). Gas exchange measurements on poikilohydric organisms were also carried out using cuvette and minicuvette systems operated in open mode (e.g., Kappen, 1983; Sancho & Kappen, 1989; Schroeter et al., 1994; Green et al., 1998; Kappen et al., 1998; Schlensog et al., 2004; Weber et al., 2012). One of the disadvantages of these devices is the need for operators, so the impossibility of performing continuous measurements in all possible environmental conditions due to constrains of the available workforce time generates bias in estimation of the primary production of these organisms by models. That is why Lange et al. (1997a) designed an automated CO_2 porometer for enabling long-term gas exchange monitoring of epilithic lichens in the field. However, although it is possible to measure

under the natural microclimatic conditions, a disadvantage of the cuvette and minicuvette systems is the necessity of releasing the samples from their original location, and in the case of terricolous organisms, from part of their substrate, to introduce them into the cuvettes.

In order to avoid any artificial alterations on the BSC samples of our climate manipulative experiment (Chapter IV) caused by the measurement procedure, the use of transparent portable chambers attached to the soil has been necessary. This implies that gas exchange of the whole soil profile is measured. This could be a disadvantage if the purpose of the study is obtaining only the net photosynthetic fluxes of the organisms belonging to the biological crust. However, within a more holistic view in which the focal point is the ecosystem functioning and its implications for the global carbon fluxes, the integration of the whole soil profile activity complements the fluxes quantification of the main components of the target ecosystem. In addition, the designed chamber allows determining the BSC gross photosynthesis with consecutive measurements in light and dark conditions, as some authors have already done (see for example Cable & Huxman., 2004; Housman et al., 2006; Freeman et al., 2009), and in Chapter IV were implemented with the support of respirometers.

A key decision is the choice of the measurement technique, i.e., an open or closed system. The open system was designed to maintain within the chamber an environment close to external conditions (Matthias et al., 1978; Ryden et al., 1978; Rolston, 1986; Mosier, 1989; Schütz et al., 1989; Gao & Yates, 1998), as for example the composition of gases in the air, the temperature and moisture of the soil and the air, etc. Hence, this system is better suited to research requiring multiple environmental treatments (Denmead, 1979), or to those in which continuous gas exchange monitoring is made in a particular sample (Hutchinson & Moiser, 1981; Mosier, 1989; Gao & Yates, 1998). However, pressure differentials caused by the continuous air flow and changes in the environmental conditions within the chamber when the reach of the steady-state takes several minutes can become problematic. Therefore, in this operation mode, the air flow into the chamber is one of the most important items in the configuration of the measurement procedure because through this variable it is possible to manage an acceptable compromise between accuracy and precision of the measurement, and the time needed to realize it. In Chapter III we show that with the volumes of the two designed chambers it is possible to measure with high air flows without causing a decrease in the accuracy (Chapter III, Figs. 3, 5). In addition, the time

required to reach the steady-state, and the tested micrometeorological alterations including changes in pressure, (Chapter III, Figs. 6, 7) were within an acceptable range.

On the other hand, one advantage of static-closed systems vs. dynamic-open systems is the simplicity, as the first do not need to measure air flows or CO_2 concentration differences between two IRGAs. This makes them more portable, cheaper, and easier to operate (Matthias et al., 1980; Rolston, 1986; Field et al., 1989; Mosier, 1989; Nakayama, 1990; Freijer & Bouten, 1991; Vourlitis et al., 1993; Gao & Yates, 1998; Lund et al., 1999). But when the aim is use a system with the versatility of both modes (open and closed), as in the case of LI-6400, this advantage disappears. Closed systems can also gain more advantage over open systems when the volumes of the chambers are becoming greater. As in closed systems the target gas does not dilute with a source of outside air, the time required to enrich the air sample with the target gas above the limit of analytical sensitivity is lower (Hutchinson & Mosier, 1981; Livingston & Hutchinson, 1995). Therefore, this method was initially more sensitive and accurate for detecting low fluxes of the target gas, but the increasing improvement of the open system technology currently offers more analytical guarantees. In our small chamber, the use of the static-closed mode offered lower accuracy when compared with a commercial dynamic-closed system, and in both chamber sizes, the relationships between closed mode and commercial systems were worse than those using open mode (Chapter III, Fig. 4c, d).

Overall, we determined that for our measurement requirements, the best configuration was the use of the chamber in open mode, with high air flow (700–800 $\mu mol\ s^{-1}$) and with and an additional fan operating at low speed if the chamber volume is higher than 2 L. Currently, the use of custom chambers for measuring gas exchange on BSCs, with or without the whole soil profile, is increasing (e.g., Grote et al., 2010; Coe et al., 2012a, b; Li et al., 2012; Su et al., 2012; Raanan et al., 2015; Wu et al., 2015), and therefore the establishment of standard methods for the acquisition of these data are needed to compare studies from different regions. Progress towards this direction is one of the goals of Chapter III.

1.2. Automated mobile rain-out shelter for rainfall manipulation experiments

Traditionally, experiments that caused shifts in temperature and/or in the concentration of CO_2 and nutrients were more common than those including precipitation alterations.

However, at least one-third of the terrestrial surface is covered by ecosystems whose functionality is constrained by water availability (Schwinning et al., 2004). As a result, the necessity of improving the modeling of climate change including feedback effects with the vegetation has increased the investment in rainfall manipulation experiments in this century. This recent interest for these kinds of experiments has promoted the emergence of a global network to assess the ecosystem sensitivity to drought (Drought-Net; www.drought-net.org) with the aim of establishing standardized methods.

Manipulation experiments involving precipitation changes have potential advantages and disadvantages. Although their temporal and spatial scales are limited, and may cause microclimatic artifacts, they also enable factor discrimination, replication, and simulation of multiple scenarios (Beier et al., 2012). These studies have been divided into two categories: passives and actives (Hanson, 2000). Passive systems consist of partial interception rain-out shelters to reduce a fixed amount of water, or complete roofs that divert water from treatment plots to be discarded or channeled to other plot with supplementary irrigation (Hanson, 2000; Gherardi & Sala, 2013). The partial interception rain-out shelter designed by Yahdjian and Sala (2002) is one of the models more replicated around the world, and has also been used in this thesis (see Chapter IV). Experimental designs that require the application of irrigation to simulate different rainfall patterns are called active precipitation manipulation experiments. This is the case of the experiment carried out in Chapters I and II, where the implementation of nine rainfall patterns required the total exclusion of natural rainfall over the experimental plots, and the application of different treatments of water quantities and irrigation schedules.

The most common environmental alterations are light attenuation and passive warming (Vogel et al., 2013), as was the case in our designed shelter for the active manipulation experiment despite having used one of the most transparent polycarbonate materials available on the market. However, its mobility allowed the exposure of the plots to the unwanted artefacts to be greatly reduced under this regime of sporadic rainfall, as it has been explained in the section of methods of Chapter I. This system was designed to be robust and resistant against adverse weather conditions such as hail and wind. The use of a robust material (double layer of transparent polycarbonate) was the cause of a greater PAR interception than expected. Therefore, this system is appropriate for sites where rainfall is scarce and the covering time of the plots is limited. This PAR reduction (50% on sunny days) may be more problematic in rainy experimental sites,

but the most challenging environmental characteristic of our study site was the frequent strong wind. We found that this system is capable of withstanding windy days, having demonstrated that it is adequate for long-term experiments.

2. Overview the importance of using multiple timescales

Ecological research needs to address processes acting across different time scales as the temporal variability of energy and material fluxes are particularly important in shaping community dynamics (Thompson et al., 2001). Some signals of this fact are that the strength of the correlation between the response magnitudes of the ecosystem processes to climate drivers suffers certain degrees of variation across different time scales (Shen et al., 2008); and that the relative importance between the effect of the natural precipitation variability on these processes and the effect of the magnitude of shifts in precipitation driven by climate change is also time scale dependent (Sala et al., 2015), since the longer the time window the lower the precipitation coefficient of variation. However, there are very few studies that use several time scales despite it seeming to be recommended. Intra- and interannual variability in rainfall drives patterns of diversity and productivity in grasslands, but their effects at interannual scale have been studied more deeply (Fay et al., 2002). Sala et al. (2015) pointed out that enhanced precipitation variability only affected soil moisture availability at intra-annual scale in grasslands with < 380 mm of rainfall per year, whereas at interannual scale, a lower level of precipitation variability had an effect on this variable in grasslands below and above 380 mm per year. Hence, ecosystem processes linked to soil moisture could be more sensitive to intra-annual changes in precipitation in arid and semiarid regions than in other areas under a wetter climate.

In this thesis, the use of different time scales have better elucidated the effects of the irrigation treatments on the *Macrochloa tenacissima*'s gas exchange variables (Chapters I and II). Net photosynthesis was most of the time unaffected by the treatments at seasonal scale, but at the annual scale it was possible to detect the negative effect of the Frequency factor, regardless of the total annual rainfall. But, on the other hand, in stomatal conductance the effects of both factors, especially frequency, were only significantly different regarding natural pattern at seasonal scale. This result showed that the plastic response of stomatal conductance and intrinsic water use

efficiency (IWUE) at seasonal scale allowed for the maintaining of photosynthetic ability under more severe precipitation regimes, whereas at annual scale these mechanisms of water control were insufficient to counteract the deleterious effect of lower and more erratic water inputs on water availability. In addition, the analysis of the ecophysiological responses at event scale was key to a better understanding of the systems processes. Dryland systems generally experience brief moments over the year of water sufficiency for the activation of all biological processes. As a consequence, ecological processes in these systems are typically described in a "pulse dynamics" framework (Noy-Meir, 1973), in which biologically significant rainfall events trigger pulses of primary production. Thus, precipitation inputs and their effects on dryland ecosystems can be rationalized as discrete events due to their large discontinuity, variability and unpredictability (Schwinning & Sala, 2004).

In Chapter IV, two survey schedules were used to glimpse different effects of the treatments: (i) bimonthly, only measuring in the early morning peak of activity; and (ii) along the whole day in the season of the greatest activity and in the start of the dormancy period. The use of both measurement strategies has allowed us to discriminate two important facts of the hydric relationships in BSCs organisms: early morning BSC activity is governed by non-rainfall water inputs (NRWI), and for this reason the indirect effect of increased temperature on the duration of NRWI had greater repercussions on photosynthesis than the reduction of rainfall. However, as the effect of NRWI is very limited in time, the duration of positive net photosynthesis along the whole day was determined by rainfall exclusion treatment when antecedent rainfalls were relevant. In conclusion, the use of different time scales offers complementary information about the effects of the treatments, helping a better understanding of the water relations of the target organisms.

3. Ecophysiological characteristics of the target organisms to cope with increased droughts and decreased rainfall frequency

Water is closely linked with the terrestrial carbon cycle. Changes in its availability for plants greatly affect their metabolic activity and carbon balance. Summer water deficit is considered the main environmental constraint for plant growth and survival in Mediterranean-type ecosystems (Galmés et al., 2007). Hence, the intensification of

droughts is one of the forecasted changes in the distribution of rainfall that could have more impact on the vegetation cover, and researches related to plant responses to water stress are becoming increasingly important (Chaves et al., 2003). Our level of understanding of the potential impacts of drought on carbon cycle, especially during the drought period, has improved greatly over the last decade. However, there are still knowledge gaps about the effect of drought for other time scales, such as during the months, or even years, after the drought period (van der Molen et al., 2011). In this thesis, the effects of increasing drought periods have been analyzed in two temporal scales: the capacity of *M. tenacissima* to recover net photosynthetic activity after the first water-pulse (Chapter II), and during the next season (Chapter I). In BSCs (Chapter IV) the timing of rainfall events remained unchanged, but the size event was reduced and its interaction with a warmer environment was analyzed.

Currently, the negative impact of the increment of drought severity, duration and/or frequency in combination with increasing temperatures is already evident in forest-ecosystem type since tree mortality is rising worldwide due to carbon starvation and hydraulic failure (Allen et al., 2010; McDowell, 2011; Choat et al., 2012). Large-scale droughts have also caused a reduction in global terrestrial net primary production (Zhao & Running, 2010). However, the studied organisms in this thesis have demonstrated a variety of physiological and morphological mechanisms to deal with prolonged drought situations, and thus a hardening of these periods will not affect their survival. In general, it is considered that perennial herbaceous plants can live long periods of severe drought because, although the water content in the bases of the leaves drops when the drought event begins, it can be stabilized with a minimum water flow when all mature leaves are senescent, allowing the survival of young tissues (Moreno et al., 2008). And the communities of organisms making up BSCs are highly specialized to withstand drought and dehydration (Zheng et al., 2011).

Previous studies have consistently reinforced the fact that *M. tenacissima* is a species with high drought resistance (Pugnaire & Haase, 1996; Pugnaire et al., 1996; Haase et al., 1999; Balaguer et al., 2002; Domingo et al., 2002; Ramírez et al., 2008a, 2009; Soumaya et al., 2014). The results of this thesis regarding the effects of lengthening days between events supports this assertion, as *M. tenacissima* had a quick response in its net photosynthetic performance to water-pulses even after three months without any rainfall. However, during summer, the most severe drought period, reducing frequency of events caused a loss of response to single water-pulses, and

during one recovery season (autumn of 2011), the negative effect of this rainfall pattern shift on net photosynthesis average was also significant. At annual scale, the response to a frequency event reduction was likewise negative. This reveals that in this species consecutive events have an additive effect until a precedent rain threshold (established in Chapter II around 150 mm in a period of 105 days), from which new rainfall events do not cause an increase in photosynthetic activity. Thus, if the number of events is lower, the sum of the responses to them will be too. This effect can be explained in a framework where a hierarchy of water-pulse events has a corresponding hierarchy of ecological responses (Schwinning & Sala, 2004), i.e., water accumulation between rainfall events leads to the creation of water-pulses of higher order, with greater infiltration depth and capacity to trigger great and longer responses. Therefore, this work has highlighted that the frequency of rainfall events over the whole year will determine the ability of *M. tenacissima* to assimilate carbon, in agreement with the increasing evidence about the great role of rainfall variability, rather than total amount, in ecosystem processes of arid, semiarid and mesic regions (Dougherty et al., 1996; Knapp et al., 2002; Harper et al., 2005; Nippert et al., 2006; Potts et al., 2006; Thomey et al., 2011; Yeager et al., 2012; Cleland et al., 2013; Peng et al., 2013).

But in BSCs, not only the frequency is critical for their annual carbon balance, but also the duration of NRWI events. For example, in Aranjuez, the experimental area where the climatic treatments have been implemented for more years, a loss of well-developed crust coverage has already been detected (Maestre et al., 2013); and it is known that in lichens, the dominant organisms of the BSCs studied in this work, the frequency of wetting and drying cycles have greater metabolic implications in their carbon balance and growth than drought duration (Lechowicz, 1981; Groulx & Lechowicz, 1987). Shifts in soil community composition are mainly driving by the inherent resistance and acclimation abilities of the different organisms to variations of these cycles, as reactivation following a dry state is costly in terms of carbon and nitrogen (Schimel et al., 2007). This thesis revealed that cyanobacteria organisms increased their ability to fix C in conditions of warmer but shorter NRWI duration, whereas the opposite occurred in covers dominated by lichens with green algae.

Stomatal conductance is the main control mechanism that plants have to face the trade-off between water loss and carbon gain. Therefore, the response of this physiological parameter must have implications for water and carbon cycles (Lauenroth & Sala, 1992). Stomatal control under low soil moisture is the major cause of low net

photosynthetic activity in plants under water stress (Ehleringer & Mooney, 1983). The stomata are closed in response to a reduction of turgor in the leaves, an increase of vapor pressure deficit in the atmosphere or by chemical signals generated in the roots (Chaves et al., 2009). Under moderate stress, a small decrease in stomatal conductance may have protective effects against stress, allowing the plant to conserve water and improve WUE (Chaves et al., 2009). Thus, it is considered that stomatal closure is among the first physiological events occurring in response to a decrease in water availability (Flexas et al., 2004). It is further known that the rapid stomatal conductance recovery after a drought event is key to achieving the total photosynthetic capacity of C_3 perennial herbaceous plants (Hu et al., 2010).

In view of the seasonal evolution of stomatal conductance and its response to the treatments in this study, *M. tenacissima* could be considered a water-spender when water availability is above the threshold of moderate water stress and a water-saver when it is below. These stomatal patterns are considered plant mechanisms to cope with water limitations using a drought avoidance strategy (Valladares et al., 2004; Chirino et al., 2011). In previous studies, the water-spender mechanism has already been detected in this species (Pugnaire & Haase, 1996; Pugnaire et al., 1996; Ramírez et al., 2008a, b; Chirino et al., 2011), but *M. tenacissima* also has morphological features corresponding to the water-saver mechanism. Its leaves are long, narrow and adapted to minimize water loss under stress conditions. Stomata are located in deep grooves on the upper side of the laminas, but the long leaves are always twisted around their axis, leaving the top underneath. In water stress situations, the leaves roll up and take on a semi to completely cylindrical shape, always with the stomata inside, limiting water loss (Pugnaire & Haase, 1996). Therefore, these anatomical traits together with the evolution of net photosynthetic activity to rates near compensation point during summer due to stomatal closure are characteristics of species with an avoidance strategy (Balaguer et al., 2002; Valladares & Sánchez-Gómez, 2006).

But, in addition to having both mechanism of a drought avoider species, the sharp drop of water potential presented in this species and its linear relationship with stomatal conductance when water availability decreases, as well as halving its leaf water content during severe droughts (Haase et al., 1999; Balaguer et al., 2002, Domingo et al., 2003), are considered traits of species with a drought tolerance strategy (Martínez-Ferri et al., 2000), since drought avoiders are normally sensitive to dehydration and need to maintain relatively high water potentials (Picon et al., 1996; Valladares et al.,

2004). Besides, drought-tolerant species are normally also photoinhibition-tolerant, as *M. tenacissima* is (Balaguer et al., 2002), i.e., they have a more pronounced photoinhibition dynamic of PSII associated with high non-radiative energy dissipation (Martínez-Ferri et al., 2000). Plants under arid climates evolve toward the development of mechanisms to avoid hydric deficit, while tolerant traits are a complement since plants are not totally impermeable to water loss and they need to tolerate a certain degree of dehydration in their tissues (Valladares et al., 2004). However, *M. tenacissima* has developed tolerance mechanisms to dehydration up to the levels of poikilohydric organisms (Balaguer et al., 2002), making it difficult to determine which of these two strategies is surrogate to the other one.

Although this species also has a quick stomatal response to changes in soil moisture content (Pugnaire et al., 1996) and down-regulated its stomatal conductance with the reduction of rainfall frequency (Chapter I, Fig. 3), a decrease of IWUE occurred with a halving in the number of events. Thus, the expected carbon acquisition with lower water loss through stomata due to the higher atmospheric CO_2 concentration (Eamus, 1991; Houghton, 2007) could be countered by this effect on IWUE in a scenario of increasing rainfall variability, as Fay et al. (2002) also noted in a mesic ecosystem. The interpretation of this signal can be framed in a context of increased competition for more and more infrequent water-pulses, as there is not adaptive advantage in maximizing the efficiency in the use of water when the water that is saved by an individual could be spent by a competitor (Valladares et al., 2004). However, a reduction of rainfall quantity triggered an increase of IWUE (Chapter I, Fig. 3), a physiological response common in Mediterranean species when they have to cope with increasing drought situations (Tenhunen et al., 1990; Ferrio et al., 2003; Medrano et al., 2009). In Chapter I, it is possible to appreciate that the up-regulation of IWUE driven by the decreasing rainfall treatments caused a greater variability in the response of *M. tenacissima* than the down-regulation of this variable driven by the decreasing frequency treatments. Therefore, although this species is opportunistic with water-pulses, and acts as a water-spender when the soil contains certain level of moisture, a conservative strategy will probably dominate the soil-plant water relationships when the periods of severe water stress increase with the decrease of rainfall quantity.

F_v/F_m is generally considered an indicator of health in photosynthetic organisms (Krause & Weis, 1991; Percival & Sheriffs, 2002; Richardson & Berlyn, 2002; Nayaka et al., 2009) as it indicates the maximal efficiency that an absorbed photon is trapped by

a reaction center of PSII (Meinander et al., 1996). Healthy, non-stressed vascular plants normally have a range of values between 0.78 and 0.86 in this parameter, with the average at 0.83 (Björkman & Demmig, 1987). However, it is usually lower in free algae and as lichen photobionts (0.63–0.78), and even more if the photobionts are cyanobacteria (0.41–0.70) as these last organisms redistribute the excitation energy in the dark in favor of photosystem I, and they possess a light-capturing pigment (phycobilin) with an invariable fluorescence yield (Papageorgiou & Gavindjee, 1968; Demming-Adams et al., 1990; Jensen, 2002). From the 29 events where F_v/F_m was measured in *M. tenacissima* (data used in Chapter I), only a maximum of two events per treatment F_v/Fm indicated a non-stress situation, and the values obtained were always 0.78–0.79, the lower extreme of the usual without-stress range. This denotes that in this ecosystem plants are subjected to one or several environmental stresses most of the time. Very likely, the dominant stress was water availability, as drylands are characterized by infrequent and variable precipitation, leading to a chronic shortage of soil moisture (Collins et al., 2014). In Chapter IV, it is possible to appreciate that BSCs were within the range of non-stress conditions only in December. But, although there was no significant F_v/F_m differences within the same BSC coverage level, high cover plots with warming treatment and low cover plots without warming treatment had an average F_v/F_m out of the non-stress range in December 2010 (Chapter IV, Fig. 6). As it has been explained in this Chapter, high biocrust cover was dominated by lichens with green-algae, whereas the activity of plots with low biocrust cover was driven by free cyanobacteria, organisms that have illustrated an opposite response to warming.

A down-regulation of F_v/F_m in water stress situations is a physiological feature of poikilohydric species (Chakir & Jensen, 1999; Augusti et al., 2001; Balaguer et al., 2002). Although this dynamic was well characterized in the BSC of this study (Chapter IV), the underlying causes of the depression in the efficiency of photosynthetic energy conversion remain unclear with our available physiological information. However, there are previous studies on cyanobacterial crusts and lichens that report some explanations and control mechanisms involved in this down-regulation. For example, the reallocation of macromolecular components, such as chlorophyll, has been seen in the epiphytic lichen *Lobaria pulmonaria* (Schofield et al., 2003). This species allocates resources toward photochemical electron generation and carbohydrate production through its greatest metabolic activity period, whereas in the less favorable period these resources are reallocated towards photoprotective mechanisms of energy dissipation. In lichen

Xanthoria parietina, seasonal changes in chlorophyll contents were also found, when the minimum and maximum contents of this pigment took place in the months with maximum and minimum irradiance, respectively (Vráblíková et al., 2006). Cyanobacterial crusts can also have temporal variation of their pigments, with a ratio of cyanobacterial frequency to chlorophyll at a concentration 2–3 times lower in fall than spring (Bowker et al., 2002). Chlorophyll loss reduces the amount of light intercepted, that means reducing the possibility of further damage to PSII by the formation of activated oxygen under high light, and increasing the ratio xanthophyll/chlorophyll concentration (Munné-Bosch & Alegre, 2000a). Xanthophyll pigments are involved in the violaxanthin/zeaxanthin cycle (= xanthophyll cycle), responsible for thermal dissipation of excess excitation energy. This cycle is present in green algae lichens, but not in cyanobacterial lichens (Heber et al., 2001). As green algae lichens are dominant in both studied sites, it is presumable that this photoprotection mechanism has played an important role in the recovery of the F_v/F_m parameter after the metabolic inactivity period.

Regarding *M. tenacissima*, despite being a vascular plant, it has the typical response to summer drought of a poikilohydric plant, characterized by a drop of chlorophyll concentration and F_v/F_m, high zeaxanthin levels, and respiration rates greater than photosynthetic activity at extremely low water potential (Balaguer et al., 2002). In this species, the role that the xanthophyll cycle plays in summer heat dissipation has been clearly described in Balaguer et al.'s (2002) study. Hence, although BSCs and *M. tenacissima* are organisms belonging to different functional groups, they share mechanisms and physiological responses to cope with the drought season, and in both cases a reduction of water availability was not a handicap to completely recover the efficiency of the PSII in autumn or winter. Chlorophyll loss as a photoprotective mechanism has been found in other Mediterranean species (Kyparissis et al., 1995; Munné-Bosch & Alegre, 2000a, b; Ain-Lhout et al., 2004; Gratani & Varone, 2004; Vitale et al., 2012), but it is not a general response to the prolonged drought period of this climatic region (Galmés et al., 2007).

Overall, in a more arid scenario, the performance and survival of *M. tenacissima*, and also community composition of BSCs, will not be mainly conditioned by more prolonged summer drought, but by the reduction of water-pulses in the rest of the year, especially in winter. Finally, we want to highlight that, in the absence of a possibility of performing a continuous gas exchange monitoring in the

experimental plots, the schedule of measurements following in this thesis (performed very near to water-inputs) can be considered the ideal for the physiological features of the target organisms, as the ecological resistance of functional poikilohydric species is not as much due to quantitative criteria of water relations, but to their capacity to reactivate the rates of photosynthesis, respiration and oxidative phosphorylation after periods of latency or low metabolic activity (Blum, 1973). In addition, in C_3 perennial herbaceous plants, the quick recovery of stomatal conductance after drought events is considered key to reach the total photosynthetic capacity (Hu et al., 2010), and particularly in *M. tenacissima*, its immediate metabolic response to water-pulses, and its water-spender behavior under or above moderate water stress, suggest that this species does not react to water-pulses with delay, unlike other woody plants with deeper roots (Schwinning et al., 2002). Besides, the rapid resumption of growth of its leaves as soon as water is available (Pugnaire et al., 1996), and the greater sensitivity to small rainfall events in summer (Chapter II) also suggest that *M. tenacissima* does not enter dormancy, being able to respond to water inputs in any time of the year. Therefore, the analysis of physiological responses using a framework of rainfall-pulses shows the high resilience of *M. tenacissima* to drought, and provides insights to the primary consequences of shifts in rainfall patterns in this kind of vegetation.

4. Climate change and variability of the physiological responses

A common objective of climate change experiments is to assess whether a climatic treatment can push the responses of the vegetation of the study site away from the current range in a context of systems in a stable state (Kröel-Dulay et al., 2015). However, ecosystems under a Mediterranean regime are most of the time subjected to transient conditions because of the great seasonal oscillations, the herbivore pressure on above-ground biomass, or the high frequency of fires. Thus, species that evolved in this dynamic equilibrium have had to adapt their physiology to these disturbance factors rather than internal regulatory mechanisms of the systems, such as density dependence or competition (Puigdefábregas, 1998). *M. tenacissima* likely has had to evolve its adaptive physiological mechanisms to an environment under conditions of dynamic systems and with a wide variability of abiotic factors. Hence, its ability to vary the physiological responses in accordance with the changing factors, especially water

availability, is very high, and it is its main strategy to cope with the great seasonal and interannual rainfall variability of semiarid Mediterranean systems.

Water reductions of the applied treatments are between the range of interseasonal range of variability, and for this reason the physiological parameters of this species had greater natural interseasonal shifts than intraseasonal shifts caused by the treatments (Chapter I, Table Appendix 1). Thus, it is expected that the strong seasonal shifts that characterize the Mediterranean climate will still be the main drivers of annual physiological patterns in *M. tenacissima*. However, the intra-annual gas exchange differences between the ambient treatment and the rainfall reduction treatment with the greater down-regulation in the second year doubled the differences between the ambient treatment in the wet hydrological year (first year) and in the hydrological year around the mean (second year). This means that the responsiveness of gas exchanges to water reduction is much greater when it takes place around the long-term rainfall average of 220 mm than around the 480 mm of the extraordinary year of precipitations. In spite of the wide average differences between the inter-treatment and natural inter-annual response variability (ITRV and IARV, respectively), the statistical results were not significant in gas exchange variables because there were fewer changes in stomatal conductance and net photosynthesis in small and medium-size plots, respectively. This could suggest that small-size plants have lower response capacity to reduce water loss in a scenario of decreasing trend of precipitations, and medium-size plants have the greatest capacity to maintain annual net photosynthesis average. Both ITRV and IARV were very low in F_v/F_m, supporting the idea that the evolution of this variable along the year in this species is governed by phenological strategies to cope with droughts rather than by direct responses to water availability.

The comparison of the variability of the natural interseasonal shifts in the physiological activity of BSCs with the variability of the intraseasonal shifts caused by the treatments was not statistically analyzed, basically because it is virtually impossible that the treatments caused higher shifts in gross photosynthesis activity than in its natural interseasonal dynamic. The reason is that, as the community of BSCs is in a state of dormancy in summer, the treatment with the greatest negative effect on gross photosynthesis should reach rates lower than 0 µmol CO_2 m^{-2} s^{-1} in order for the response variability between treatments to surpass the differences between the maximum and minimum activity of control plots throughout the year. However, although in both functional groups the ranges of shifts caused by the treatments were

not greater than the current natural interseasonal shifts, it did not mean that the effects of the treatments could not surpass the resistance of change of these organisms. Most probably, the survival of BSCs organisms to rainfall shifts are not endangered at short time scales (i.e., intraseasonality) due to the great adaptation of their metabolism to water availability, but as the opportunities that they have to fix carbon are very limited in time in dryland ecosystems, a further reduction of these periods can be fatal for their survival along longer time scales. Examples of this effect can be found in Coe et al. (2012b) and Maestre et al. (2013). In conclusion, due to the great representativity of both *M. tenacissima* and BSCs in semiarid areas of the western Mediterranean, the study of the physiological ranges of these communities under different rainfall patterns is key to glimpse the trends of these ecosystems in a scenario of climate change.

5. Insights of the response thresholds to water-pulses

Ecologically effective precipitation pulse has been defined as a rainfall event that generates enough alterations in soil and plant water status, resulting in discernible variations of ecosystems-atmospheric CO_2 fluxes (Hao et al., 2012). Its threshold has been set at 5 mm in a semiarid steppe (Hao et al., 2013). Although this thesis has been focused on plant or biological community scales, the results have clear implications at the ecosystem scale. The importance of this threshold has only been observed in *M. tenacissima* in summer (Chapter II), when the soil moisture is minimal. Not only the size, but also the timing of the event is a main feature that determines the magnitude of the biological responses in Mediterranean and dryland ecosystems (Austin et al., 2004; Sher et al., 2004; Bates et al., 2006). Thus, the thresholds of effective rainfalls are modulated by seasonality and the soil moisture configured by the antecedent rainfall features. In summer, *M. tenacissima* was especially sensitive to small events, and the 5 mm threshold was not the limit where a significant response was detected, but where the response suffered a change in its signal. This species, due to its shallow roots and its capacity to respond quickly to rainfall pulses, had a significant response to < 5 mm events in summer, but in a negative direction as they likely promoted photorespiration rather than photosynthesis. Nevertheless, when the event size exceeded this threshold, net photosynthesis could be significantly increased. In the rest of the seasons the response of net photosynthesis to water-inputs was far more conditioned by the features

of the antecedent rainfall, and hence, obtaining a single threshold of effective rainfall is not possible. But presumably, due to the opportunistic nature of this species, soil moisture and the threshold of effective rainfall are positively correlated.

The threshold of effective rainfall event is also conditioned by the organism type. For example, BSCs can activate their metabolisms with less than 1 mm of precipitation equivalent (Lange et al., 1997b, 1998; Lange, 2003), and therefore they can use another source of water, such as dew or fog (Lange et al., 1992, 1994; Veste et al., 2001). This fact was highlighted in Chapter IV, as the responses to a climate change scenario were mainly driven by the availability and duration of NRWI. The importance of these types of water-pulses was also emphasized in other regions, as for example Huang et al. (2014) estimated that carbon fixation through NRWI is supposed to be between 30 and 44% of the total fixation of these organisms in a sandy dryland; or even near to 100% in a coastal fog desert (Lange et al., 1994, 2006). But, as noted above, the characteristics of NRWI (e.g., the timing and duration of the soil surface wetting) also condition the signal of the responses, as if the soil loses the moisture before light intensity allows photosynthesis, the pulses only activate dark respiration and promote a negative balance instead of a positive one via carbon fixation (Lange, 2003). Although the dominant organisms of the experimental BSCs have a high optimal temperature to fix carbon, between 25 and 38ºC (Lange et al., 1997b, 1998; Lange & Green 2004; Pintado et al., 2005), they get dry before they begin the carbon fixation if the temperatures rise, a phenomenon that occurs most often when rainfall is less than 1 mm (Su et al., 2012). Indeed, an increase of small rainfall events in summer can lead to the death of the BSC dominant organisms (Reed et al., 2012). In addition to developed BSCs, microbial communities in the upper profile of soils also mediate carbon exchanges between soil and the atmosphere, responding to small events of minute duration or that infiltrate only 1–2 cm into soils (Huxman et al., 2004; Collins et al., 2014). As in BSCs dominated by lichens, very small events only trigger the activity of heterotrophic organisms, releasing carbon to the atmosphere, since the autotrophic free cyanobacteria require greater water quantity, and always in a liquid state (Lange et al., 1986; Farquhar et al., 1989), to reach net photosynthesis (Belnap et al., 2004; Huxman et al., 2004; Bowling et al., 2011). But, unlike BSCs, free cyanobacteria have physical and physiological characteristics (mentioned in Chapter IV) that favor their ability to fix carbon under a regime of lower NRWI and a warmer environment (Chapter IV, Fig. 6).

Ecosystem memory to past precipitation events is a critical feature of ecosystem functioning in arid and semiarid areas (Paruelo et al., 2005). The memory of annual rainfall can influence the productivity of the next year, but in recent rainfall events it establishes the basis for ecosystem sensitivity to intraseasonal precipitation patterns (Schwinning et al., 2004). In this way, the level of soil moisture of upper-medium layers is determined by recent precipitations that may dampen or amplify the effect of a new individual precipitation pulse on plants with shallow root systems (Reynolds et al., 2004). For example, *M. tenacissima* was more sensitive to the size of water-pulses in situations of proximity to soil water depletion, whereas in the peak of the photosynthetic rates were mainly determined by the quantity and frequency of rainfalls over 105 days (Chapter II, Fig. 7). In addition, in this thesis a clear linkage between the rainfall attributes of the antecedent conditions has been found in early spring. When accumulated rainfall during the precedent 105 days was lower than 75 mm, the frequency of the events in the same period of time did not affect the photosynthetic rates of *M. tenacissima*, but surpassing this threshold of accumulated rainfall, the ability of this species to fix carbon increased with the number of rainfall events (Chapter II, Fig. 7f). Hence, as Potts et al. (2006) also demonstrate, the ability of semiarid grassland ecosystems to translate soil moisture into assimilated carbon during the growing season is greatly influenced by the characteristics of the antecedent rainfall rather than by the size of the events. In Chapter IV, it was also possible observe different responses to the treatments due to the characteristics of the antecedent rainfalls in the whole day surveys. In Sorbas, the whole day measurements were performed a few days after a rainfall event of 11 mm, and the antecedent rainfall of the month was 54 mm. In Aranjuez, it only rained 2 mm prior to the measurements and no more rainfall events took place during the month. In the plots of Sorbas that received all the natural rainfall, positive net photosynthesis was possible all day, whereas the 30% reduction of rainfall supposed the prevalence of respiratory activity at midday (Chapter IV, Fig. 8). However, in Aranjuez, positive net photosynthesis only occurred in the early morning in the plots without the drying effect of the warming treatment.

The response thresholds to water pulses were also mediated by the size of *M. tenacissima* tussocks. Other studies support the results obtained in this experiment using the same species (Armas & Pugnaire, 2005; Ramírez et al., 2008a, b) or others of dryland environment (Caldwell et al., 1983; De Soyza et al., 1996). As small plants have less developed root system in depth, and they need to designate more carbon

resources to grow, they have higher net photosynthetic rates than larger plants in winter and early spring, which could be an advantage in an intraspecific and interspecific competition with annual plants for water resources in the upper soil layer, as in this kind of ecosystem they have their peak of productivity in the mentioned seasons. On the other hand, larger plants had higher net photosynthetic rates at reduced soil water and high light stress, a characteristic also found in other tussock species (Caldwell et al., 1983), and in a desert shrub (Soyza et al., 1996).

6. The role of drylands in the carbon cycle under a global change scenario

Because of the vast areas that drylands represent, and the importance of their soil carbon pools, these regions could have a strong impact on the global carbon cycle, although the extent to which they modulate global atmospheric CO_2 levels is still poorly understood and less studied than other biomes, such as humid tropics and Arctic regions, because they seem to have low rates of biological activity and carbon stock mobility (Wohlfahrt et al., 2008; Schimel, 2010). However, land degradation, particularly desertification, in drylands is being accelerated by global change, often resulting in a balance of carbon emission to the atmosphere (Lal, 2004), so appropriate management of these regions is key to enhance carbon fluxes in the right direction (to biological and soil carbon sinks). There are evidences that BSCs contribute to increasing soil carbon stocks, as their growth increases the organic carbon content of the topsoil (Gypser et al., 2015). Although the range of surface-area-related net photosynthesis is similar to typical higher plants under optimal conditions (Lange et al., 1994), BSCs productivity in arid and semiarid regions is restricted to short periods of wetting by sporadic rainfall or NRWI that disrupt the dominant dormancy state of the desiccated thallus (Lange et al., 1997b). Therefore, with very few exceptions, photosynthesis performance in BSCs is mainly determined by water availability (Lange et al., 2001).

The same strong relationship is possible to formulate with *M. tenacissima*, as its gas exchange and short-term growth is dependent on the current water status of the soil (Pugnaire et al., 1996). As we have shown in this thesis, the reduction of the frequency of effective rainfalls to trigger metabolic activity can cause a loss of potential of these organisms to fix carbon, and as these two target organisms are the most representative

vegetation of the studied areas, they will likely be major players involved in driving these ecosystems towards acting as carbon sources. Balsa Blanca, one of the studied areas, is currently recognized as a carbon source (Serrano-Ortiz et al., 2014), and the forecasted climate trends seems to reinforce this status. However, if the rainfall frequency decrease is accompanied by an increase in the size of the event, its negative effect on carbon fixation can be reduced or neutralized in *M. tenacissima*, as it has been seen in Chapter II. In addition, this species has a plastic response to environment situations, maximizing the carbon fixation whenever the conditions for it are appropriate (Pugnaire & Haase, 1996). Therefore, semiarid areas with vegetation very opportunistic to water pulses could be especially sensitive to favorable hydrological years, acting as carbon sinks. Recently, the use of an ensemble of ecosystem and land-surface models, as well as empirical observations of global gross primary production, have allowed the discovery that biogeographic regions have different roles in the terrestrial carbon sink (Ahlström et al., 2015). This study shows that the mean carbon sink is primarily determined by highly productive lands, but the trend and interannual variability of this sink are driven by the response of semiarid ecosystems to climate. As is expected, positive gross primary production anomalies in these regions are dominated by cool and wet conditions, whereas negative gross primary production anomalies are driven by warm and dry conditions. Therefore, a deeper understanding of the processes involved in carbon balances of semiarid regions seems to be key to evaluate the trends of carbon sequestration at a global scale; and in conclusion, the results of this thesis aim to be a contribution of global scale significance in determining the role of dryland regions in the carbon cycle.

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General conclusions

GENERAL CONCLUSIONS

1. The use of different time scales in both Macrochloa tenacissima and BSCs allowed for perceiving different effects of the simulated climate change, demonstrating that the study of physiological and ecological processes requires different time approaches for a better understanding of them (Chapters I, II and IV).

2. The rainfall reduction treatments only caused a seasonal decrease of net photosynthetic rates of M. tenacissima in the last experimental year. This was possible due to the adaptability of the water control mechanisms to different soil moisture situations through stomatal conductance and intrinsic water use efficiency (IWUE), which changed their rates in response to the treatments from the first experimental summer. These regulatory mechanisms to maintain the levels of photosynthesis under higher water stress regimes were effective at the seasonal scale, but at the annual scale, the reductions of rainfall frequency caused a drop in the rates of this variable (Chapter I).

3. At the event scale, the lengthening of days without precipitation did not affect the quick response of M. tenacissima to water-pulses, and the intensification of the events (i.e., increase of the event size in the regime of lower rainfall frequency) caused a greater instantaneous responses to water-pulses in its net photosynthesis. But as in the upper temporal scales prevailed a negative effect of frequency reduction of precipitation on this variable, the durations of these responses were not likely enough to be sustained until the next rainfall in an scenarios of lower events, and therefore did not trigger responses of superior order (Chapters I and II).

4. Intraseasonal physiological responses of M. tenacissima to the experimentally simulated future rainfall patterns did not exceed their current range of interseasonal variability due to the strong seasonal shifts of the Mediterranean climate, showing it is a well drought-adapted species. However, annual rainfall decreases regarding the long-term average, together with a concentration of the events, caused greater down-regulations of gas exchange variables (net photosynthesis and stomatal conductance) than up-regulations when higher annual numbers of rainfall events and a similar magnitude of rainfall quantity variations took place over the long-term precipitation

average. Thus, the forecasted rainfall change will very likely lead this species to exceed their current long-time response ranges (Chapter I).

5. The chamber designed to measure net photosynthesis on BSCs has shown consistent values in its comparison with two different respirometer brands when it was operating in an open system (air within the system is renewed) with high air flow and an additional fan if the volume of the chamber was over 2 L, validating this method and enabling the *in situ* monitoring of net photosynthesis without disturbing the BSC, and the estimation of gross photosynthetic rates by its simultaneous use with a respirometer (Chapter III).

6. BSC hydration status was the main environmental factor controller of its physiological parameters, reflecting the strong seasonal fluctuations that characterize the Mediterranean climate, and allowing it a complete daily activity only in situations of optimal hydration (Chapter IV).

7. The BSC photosynthesis in the early morning was governed by non-rainfall water inputs (NRWI), and therefore they modulated the BSCs physiological responses to a warmer climate at dawn. Warming did not have a direct effect on photosynthesis, but indirectly decreased the formation of dew on BSCs and accelerated their dehydration. Therefore, in regions where dew is a main source of water-pulses, BSCs will lose metabolic activity time and their ability to sequester carbon will be reduced. Forecasting the warming effects on BSCs in regions where fog is the main supply of water is more complicated. Both local factors and global processes condition the formation and circulation of fog, and therefore there is a great uncertainty of how climate change will impact on this NRWI (Chapter IV).

8. The negative impact of rain reduction was only detected in a whole day survey after abundant preceding precipitations. As in drylands this situation is more restricted in time than NRWI pulses, the impact of rainfall amount decrease will be secondary to the effects that a NRWI decrease could have in the annual carbon balance of these organisms (Chapter IV).

9. Early-successional cyanobacterial BSCs could displace more developed lichen-dominated biocrusts in Mediterranean drylands due to the energetic advantages of

their photosynthetic system under a warmer environment and their mechanisms to slow down or avoid desiccation (Chapter IV).

10. The target organisms, belonging to the two dominant biotypes and together representing one of the most widespread ecosystems in the Mediterranean semiarid environment, can resist great levels of dehydration, so the forecasted lengthening of summer droughts will not likely affect their capacity for metabolic recovery. However, they will be more sensitive to the frequency and duration of the water-pulses during the rest of the year, and a decrease of water-inputs will reduce their periods of high carbon fixation (Chapters I, II and IV).

Conclusiones generales

CONCLUSIONES GENERALES

1. El uso de diferentes escalas de tiempo tanto en Macrochloa tenacissima como en las CBSs permitió percibir diferentes efectos de la simulación de cambio climático, demostrando que el estudio de los procesos fisiológicos y ecológicos requiere diferentes aproximaciones temporales para un mejor entendimiento de ellos (Capítulos I, II y IV).

2. Los tratamientos de reducción de precipitación solo causaron a escala estacional un descenso de las tasas fotosintéticas de M. tenacissima en el último año experimental. Esto fue posible debido a la adaptabilidad de los mecanismos de control del agua a diferentes situaciones de humedad del suelo a través de la conductancia estomática y de la eficiencia intrínseca en el uso del agua (EIUA), las cuales cambiaron sus tasas en respuesta a los tratamientos desde el primer verano experimental. Estos mecanismos regulatorios para mantener los niveles de fotosíntesis en regímenes de alto estrés hídrico fueron efectivos a escala estacional, pero no a escala anual, ya que las reducciones de la frecuencia de lluvia causaron una caída de las tasas en esta variable (Capítulo I).

3. A escala de evento, la prolongación de los días sin precipitación no afectó a la rápida capacidad de respuesta de M. tenacissima a los pulsos de agua, y la intensificación de los eventos (esto es, el incremento del tamaño de evento en un régimen de menor frecuencia de lluvia) provocó que su fotosíntesis neta tuviera una mayor respuesta instantánea a los pulsos de agua. Pero como en las escalas temporales superiores prevaleció un efecto negativo de la reducción de frecuencia de precipitaciones en esta variable, probablemente las duraciones de estas respuestas no fueron suficiente para ser mantenidas hasta la siguiente lluvia en un escenario de menor número de eventos, y por ello no desencadenaron respuestas de ordenes superiores (Capítulos I y II).

4. Las respuestas fisiológicas intra-estacionales de M. tenacissima a los futuros patrones de lluvia experimentalmente simulados no excedieron sus rangos actuales de variabilidad interestacional debido a los fuertes cambios estacionales del clima Mediterráneo, mostrando que es una especie bien adaptada a la sequía. Sin embargo, el descenso de las precipitaciones respecto al promedio a largo plazo, junto con un

incremento de las concentraciones de los eventos, causó una regulación a la baja en las variables de intercambio gaseoso (fotosíntesis neta y conductancia estomática) de mayor magnitud que la regulación al alza cuando tuvo lugar un mayor número anual de eventos de lluvia y las variaciones de la cantidad de lluvia fueron similares al promedio de precipitaciones a largo plazo (Capítulo I).

5. La cámara diseñada para medir fotosíntesis neta en CBSs ha mostrado valores consistentes en su comparación con dos marcas de respirómetros diferentes cuando fue configurada en sistema abierto (el aire dentro del sistema se renueva) con un flujo de aire alto y un ventilador adicional si el volumen de la cámara fue mayor a 2L, validando este método y permitiendo el seguimiento *in situ* de la fotosíntesis neta sin alterar la CBS, y la estimación de las tasas de fotosíntesis brutas mediante su uso simultáneo con un respirómetro (Capítulo III).

6. El status hídrico de la CBS fue el principal factor ambiental controlador de sus parámetros fisiológicos, reflejando las fuertes fluctuaciones estacionales que caracterizan al clima Mediterráneo, y permitiendo la actividad de la CBS durante todo el día únicamente en situaciones de hidratación optimas (Capítulo IV).

7. La fotosíntesis de la CBS a primera hora de la mañana fue gobernada por las precipitaciones ocultas, y por ello este aporte de agua moduló las respuestas fisiológicas de las CBSs al calentamiento climático al amanecer. Dicho calentamiento no afectó directamente a la fotosíntesis, pero sí indirectamente mediante la disminución de la formación de rocío sobre las costras y acelerando su deshidratación. Por lo tanto, en regiones donde el rocío es la principal fuente de agua, las CBSs perderán tiempo de actividad metabólica y su habilidad para secuestrar carbono se verá reducida. Predecir los efectos del calentamiento sobre las CBSs en regiones donde la niebla es la principal fuente de agua es más complicado. Tanto factores locales como procesos globales condicionan la formación y circulación de la niebla, y por ello hay un elevado grado de incertidumbre en el impacto que tendrá el cambio climático en este tipo de precipitación oculta (Capítulo IV).

8. Sólo se detectó el impacto negativo de la reducción de la lluvia en un muestreo de día completo tras de un mes de abundantes precipitaciones. Como en los sistemas limitados por el agua esta situación está más restringida en el tiempo que los pulsos de precipitaciones ocultas, el impacto de la disminución de la cantidad de lluvia será

secundario a los efectos que la disminución de las precipitaciones ocultas pudieran tener en el balance anual de carbono en estos organismos (Capítulo IV).

9. Las CBSs de cianobacterias de sucesión temprana podrían desplazar a las costras más desarrolladas dominadas por líquenes en las zonas secas del Mediterráneo debido a las ventajas energéticas de sus sistemas fotosintéticos bajo un ambiente más cálido y a sus mecanismos para ralentizar o evitar la desecación (Capítulo IV).

10. Los organismos objeto de estudio, pertenecientes a los dos biotopos dominantes y representando juntos a uno de los ecosistemas más extendidos en el ambiente semiárido Mediterráneo, pueden resistir grandes niveles de deshidratación, por lo que la prolongación de las sequías estivales pronosticada probablemente no afectará a su capacidad de recuperación metabólica. Sin embargo, serán más sensibles a la frecuencia y duración de los pulsos de agua durante el resto del año, y una reducción de las entradas de agua al ecosistema disminuirá sus periodos de alta fijación de carbono (Capítulos I, II y IV).

Summary

SUMMARY

Climate change is a component of the several planetary-scale shifts that are currently taking place in the Earth system, which as a whole are referred to as global change. However, climate, as a vector of energy and material fluxes, has the ability to interact with all the elements (including natural and social components) of the Earth system, also influencing their dynamics. The Mediterranean-European region has been identified as one of the most prominent climate response hot spots, and precipitation patterns are major limiting factors for human activities and natural ecosystems in this area. Climate model projections forecast an increase of 4–5°C over the course of this century in the Mediterranean-European region, and although with less confidence in specific values, they also detect a robust signal of change in precipitation patterns. These changes will consist of a decrease in the amount and frequency of precipitation, and an intensification of extreme precipitation events.

Macrochloa tenacissima is a rhizomatous, C_3 perennial tussock grass widespread and endemic in Western Mediterranean drylands, and is one of the few species that is usually dominant in its community. The soil in the interspaces of these tussocks is frequently covered by biological soil crust (BSC or biocrust), a community of organisms made up of cyanobacteria, green algae, heterotrophic bacteria, microfungi, lichens and bryophytes. Therefore, the major goal of this thesis is to estimate the effects of climate change on the ecophysiology of these two dominant functional types of the semiarid Western Mediterranean region. For this purpose, after a **general introduction** where we show the current background of this topic and address the specific goals, this thesis has been structured in the following chapters:

In **Chapter I**, a rainfall manipulation experiment in field conditions was conducted on *M. tenacissima* during a three-year period to test the effects of the full-crossing interaction of two factors: quantity and frequency of rainfall, both at three levels (100%, 75% and 50% of natural rainfall). Using a mobile rain-out shelter, all natural rainfall was removed from the experimental plots, and by means of manual irrigation, the nine different treatments were carried out. The physiological responses of this species to the treatments were analyzed at the seasonal and annual scales, obtaining in the gas exchange variables (net photosynthesis and stomatal conductance) different results along these time ranges. Net

photosynthesis response to the treatments was stronger at the annual scale than at the seasonal, whereas in stomatal conductance, the strongest response took place at the seasonal scale. In both gas exchange variables, frequency factor was the main driver of the response. The decrease in rainfall quantity and frequency had opposite effects on intrinsic water use efficiency, enhancing and diminishing it, respectively. Rainfall reduction treatment did not decrease the maximum photochemical efficiency of photosystem II (F_v/F_m) regarding ambient treatment. In conclusion, this species showed that it possesses a great resistant photosynthetic apparatus to water stress and a very plastic response to water-pulses.

In **Chapter II**, using the same rainfall manipulation experiment as in the previous chapter, the response of *M. tenacissima* net photosynthesis to different rainfall attributes was analyzed. Seasonality, acting both through water availability and plant phenology, played a main role in the relations of this species with rainfall features, as the results obtained support: (i) the magnitude of antecedent precipitations in an interval of 105 days had greater importance on net photosynthesis rates in early spring; (ii) in late spring, a sharp down-regulation of net photosynthesis took place, independently of the antecedent precipitations or the magnitude of the last event; (iii) however, summer was the season with the greatest sensitivity to the size of the preceding event. The discrimination between the effect caused by increasing drought periods occurring between events and reducing rainfall frequency in a greater scale of time was also tested. The increase of temporal separation between two events did not reduce the response capacity of *M. tenacissima* to water-pulses if a size increase of the last event took place; however, the frequency decrease of events quantified during 105 days had a negative impact on net photosynthesis. Finally, we also found different responses depending on the plant size in all seasons. Larger plants had higher carbon fixation capacity in harsh environmental situations, whilst small and medium size plants had higher rates under favorable conditions for photosynthetic activity.

In **Chapter III**, we described the process of designing and testing two versions of a custom chamber with different volumes for measuring *in situ* BSC carbon exchange. To compare the measurements of our custom chamber (attached to a LI-COR 6400) with commercial chambers, we used three reference systems: two respirometers (LI-COR 8100 and EGM-4, PP-Systems); and a commercial conifer chamber (6400-05, Li-COR Inc.),

attached to another LI-COR 6400, to also test the response of the chambers at a positive range of net photosynthesis. Carbon fluxes with the best correlations regarding reference systems were determined by the designed chamber (i) operating as an open system; (ii) with an airflow between 700–800 μmol s^{-1}; (iii) with an additional fan with a speed of 0.7 m s^{-1} in the large chamber, and without it in the small chamber; and (iv) with the sample IRGA internal fan set to a fast position. Our results verified that the methodology proposed was consistent and appropriate for use in the field, without significantly altering the micro-environmental condition of the samples.

In **Chapter IV**, a climate manipulative full factorial experiment in two representative dryland ecosystems (in Sorbas, Almería, and in Aranjuez, Madrid) was conducted during a one and a half year period to evaluate how precipitation, temperature and BSC cover affect the assimilation and net carbon balance of BSC communities. Each factor had two levels: natural precipitation and reduced ~ 30% (using passive rain-out shelters), natural temperature and increased 2–3ºC on average on an annual basis (using open top chambers), poorly developed BSC communities with cover < 25% and well-developed BSC communities with cover > 50%. In Sorbas, warming reduced the BSC carbon fixation at early mornings throughout the year by decreasing the quantity and duration of dew on the soil surface. In Aranjuez, where fog was the main non-rainfall water input (NRWI), net photosynthesis in the highest period of activity was significantly greater in high biocrust cover plots under natural conditions and in the rainfall reduction treatment. During full daily surveys performed in winter, rainfall reduction treatment decreased BSC carbon fixation in Sorbas, where antecedent monthly precipitation was relevant; whereas in Aranjuez, where the antecedent monthly precipitation was negligible, warming was the factor that decreased BSC performance. In conclusion, early BSC photosynthesis was governed by NRWI, whereas rainfall reduction treatment determined the duration of positive net photosynthesis throughout the day, when preceding precipitation was relevant.

In the **general discussion**, we comment on the advantages and disadvantages of two custom devices designed and implemented for this thesis, as well as other methodological issues related to the experiments, and we emphasize the main overall results, which are contextualized in their theoretical framework. This section can be summarized as follows:

1. We discussed the features and functioning of (i) an automated mobile rain-out shelter for excluding all the natural rainfall without causing significant micrometeorological alterations on experimental plots (described in Chapter I and used in Chapters I and II); and (ii) a portable chamber for measuring gas exchange on BSCs without removing them from their natural growth place (described in Chapter III and used in Chapter IV). Both devices have shown satisfactory results.
2. We discussed the relationship between ecophysiological responses and time scales, and stated: (i) The statistically significant responses to the climatic factors changed with the time scale. Thus, including different time scales is essential to understanding the ecological processes, and to unravelling the water relations of the target organisms. (ii) The current variability of the ecophysiological responses is key to assessing the real impact of the climate change. (iii) The ecosystem memory to past precipitation events is a critical feature of semiarid ecosystem functioning, and the influence of the antecedent rainfall characteristics on ecophysiological responses must be assessed.
3. We have established thresholds of water inputs triggering ecophysiological responses. In *M. tenacissima*, seasonality and the soil moisture configured by the antecedent rainfall features modulated the thresholds of effective rainfalls. This species was more sensitive to small water-pulses in summer, promoting respiration and/or photorespiration when rainfall was < 5 mm, and increasing net photosynthesis in larger events. BSCs can activate their metabolisms with NRWI lower than 1 mm of precipitation equivalent, but if the hydration does not persist until BSC receives enough light, the activation of dark respiration promotes a negative carbon balance.
4. We discussed the responses of the target organisms to the forecasted climate change. *M. tenacissima* used both tolerance and avoidance strategies to cope with water stress. As poikilohydric organisms, BSCs were dormant in the periods of greater water stress. Both target organisms can resist great levels of dehydration and show high resilience, so an increase of the drought period will not likely affect their recovery capacity. However, they will be more sensitive to the frequency and duration of the water-pulses during the no-drought periods, especially in winter, and a decrease of rainfall quantity will reduce their time of carbon fixation.

5. We finally discussed the role of drylands in the carbon cycle under a global change scenario. Our review of the most recent literature shows that: (i) one of the studied areas in this thesis is currently a carbon source and (ii) the trend of interannual variability of global carbon sink is driven by the response of semiarid ecosystems to climate; and (iii) the on-going climate change will likely increase the semiarid areas acting as carbon sources.

Finally, a section of **general conclusions** is included at the end of this report with the ten highlights of the thesis.

Resumen

RESUMEN

El cambio climático es un componente de los diversos cambios a escala planetaria que actualmente están teniendo lugar en el Sistema tierra, los cuales en su conjunto son referidos como cambio global. Sin embargo, el clima, como vector de flujos energía y materia, tiene la habilidad de interactuar con todos los elementos (incluyendo componentes naturales y sociales) del Sistema tierra, influenciando así también en sus dinámicas. La región Mediterránea de Europa ha sido identificada como uno de los puntos calientes de respuesta al cambio climático más relevantes, y en esta área los patrones de precipitación son los principales factores limitantes para la actividad humana y los ecosistemas naturales. Las proyecciones de los modelos climáticos predicen un incremento de temperatura de 4–5ºC durante el curso de esta centuria en la región Mediterránea de Europa, y aunque con menor fiabilidad en un valor específico, también detectan una señal de cambio robusta en los patrones de precipitación. Estos cambios consistirán en una disminución de la cantidad y frecuencia de la precipitación, así como una intensificación de los eventos de precipitación extremos.

Macrochloa tenacissima es una planta C_3 herbácea perenne, cespitosa y rizomatosa ampliamente distribuida y endémica de las zonas secas del Mediterráneo Occidental, y es una de las pocas especies que normalmente es dominante en su comunidad. El suelo presente entre las macollas de esta especie está frecuentemente cubierto por costra biológica del suelo (CBS o biocostra), una comunidad de organismos compuesta por cianobacterias, algas verdes, bacterias heterotróficas, microhongos, líquenes y briófitos. Por lo tanto, el principal objetivo de esta tesis es estimar los efectos del cambio climático en la ecofisiología de estos dos tipos funcionales dominantes en la región semiárida del Mediterráneo Occidental. Para este propósito, después de la **introducción general** donde mostramos el actual estado de conocimiento de este tema y abordamos los objetivos específicos, esta tesis ha sido estructurada en los siguientes capítulos:

En el **Capítulo I**, se ha realizado un experimento de manipulación de lluvias usando como especie objetivo *M. tenacissima* durante tres años para testar los efectos de dos factores completamente cruzados: cantidad y frecuencia de lluvia, ambos con tres niveles (100%, 75% y 50% respecto a la lluvia natural). Usando una cubierta móvil de exclusión de

lluvia, se eliminó toda la lluvia natural de las parcelas experimentales, y mediante riegos manuales, se llevaron a cabo los nueve tratamientos diferentes. Las respuestas fisiológicas a los tratamientos en esta especie fueron analizadas a escalas estacionales y anuales, obteniendo en las variables de intercambio gaseoso (fotosíntesis neta y conductancia estomática) diferentes resultados a lo largo de estos rangos temporales. La respuesta de la fotosíntesis neta a los tratamientos fue más fuerte en la escala anual que en la estacional, mientras que en la conductancia estomática la respuesta más fuerte tuvo lugar en la escala estacional. En ambas variables de intercambio gaseoso, el factor frecuencia fue el principal impulsor de la respuesta. La disminución en la cantidad y frecuencia de las precipitaciones tuvo efectos opuestos en el uso eficiente del agua intrínseco, incrementándolo o reduciéndolo, respectivamente. El tratamiento de reducción de lluvia no redujo la eficiencia fotoquímica máxima del Fotosistema II (F_v/F_m) respecto a los valores obtenidos en condiciones naturales. En conclusión, esta especie mostró poseer un aparato fotosintético muy resistente al estrés hídrico y respuestas muy plásticas a los pulsos de agua.

En el **Capítulo II**, usando el mismo experimento de manipulación de lluvia que en el capítulo anterior, se analizó la respuesta de la fotosínteis neta de *M. tenacissima* a diferentes atributos de la lluvia. La estacionalidad, actuando tanto a través del agua disponible como de la fenología de la planta, jugó un papel principal en las relaciones de esta especie con las características de la lluvia, ya que los resultados obtenidos sostienen que: (i) la magnitud de las precipitaciones antecedentes en un intervalo de 105 días tuvieron una mayor importancia en las tasas de fotosíntesis neta en la primavera temprana; (ii) en la primavera tardía se produjo una fuerte regulación a la baja de la fotosíntesis neta, independientemente de las lluvias antecedentes o de la magnitud del último evento; (iii) sin embargo, el verano fue la estación con mayor sensibilidad al tamaño del evento precedente. También se realizó una discriminación entre el efecto causado por un incremento de los periodos de sequía entre eventos y la reducción de la frecuencia de las lluvias a una escala temporal mayor. El incremento de la separación temporal entre dos eventos no redujo la capacidad de respuesta de *M. tenacissima* a los pulsos de agua si tuvo lugar un incremento del tamaño del último evento; sin embargo, la reducción de la frecuencia de eventos cuantificados durante 105 días tuvo un impacto negativo en la fotosíntesis neta. Finalmente, también encontramos diferentes respuestas en función del tamaño de la planta en todas las

estaciones. Las plantas de mayor tamaño tuvieron mayor capacidad de fijar carbono en situaciones ambientales adversas, mientras que las plantas de tamaño pequeño y mediano tuvieron mayores tasas bajo condiciones favorables para la actividad fotosintética.

En el **Capítulo III**, describimos el proceso para diseñar y testar dos versiones de diferentes volúmenes de una cámara hecha a propósito para medir el intercambio gaseoso *in situ* de la CBS. Para comparar las mediciones de nuestra cámara (acoplada a un LI-COR 6400) con las cámaras comerciales, usamos tres sistemas de referencia: dos respirómetros (LI-COR 8100 y EGM-4, PP-Systems); y una cámara comercial para coníferas (6400-05, Li-COR Inc.), acoplada a otro LI-COR 6400, para testar también la respuesta de las cámaras en rangos positivos de fotosíntesis neta. La cámara diseñada determinó los flujos de carbono con las mejores correlaciones respecto a los sistemas de referencia (i) operando en sistema abierto; (ii) con un flujo de aire entre los 700–800 μmol s^{-1}; (iii) con un ventilador adicional en la cámara grande funcionando a una velocidad de 0.7 m s^{-1}, y sin él en la cámara pequeña; y (iv) con el ventilador interno del IRGA muestra fijado a velocidad rápida. Nuestros resultados verificaron que la metodología propuesta fue consistente y apropiada para su uso en el campo, sin que produjera una alteración significativa de las condiciones microambientales de las muestras.

En el **Capítulo IV**, se realizó un experimento manipulativo del clima factorial completo en dos ecosistemas semiáridos representativos (en Sorbas, Almería, y en Aranjuez, Madrid) durante un periodo de año y medio para evaluar cómo la precipitación, temperatura y la cubierta de CBS afectan a la asimilación y al balance de carbono neto en las comunidades de CBS. Cada factor tuvo dos niveles: precipitación natural y reducida ~ 30% (usando una cubierta de exclusión de lluvia pasiva), temperatura natural e incrementada 2–3ºC en promedio anual (usando cámaras abiertas en su parte superior), comunidades de CBS poco desarrolladas con una cobertura < 25% y comunidades de CBS bien desarrolladas con coberturas > 50%. En Sorbas, el calentamiento redujo la fijación de carbono de la CBS a primeras horas de la mañana a lo largo del año debido a la disminución de la cantidad y duración de rocío en la superficie del suelo. En Aranjuez, donde la niebla fue la principal precipitación oculta, la fotosíntesis neta en el periodo de mayor actividad fue significativamente mayor en las parcelas con alta cobertura de CBS bajo condiciones naturales y con tratamiento de reducción de las precipitaciones. Durante

los muestreos de días completos realizados en invierno, el tratamiento de reducción de precipitaciones disminuyó la fijación de carbono de la CBS en Sorbas, donde la lluvia mensual antecedente fue relevante; mientras que en Aranjuez, donde la lluvia mensual antecedente fue muy reducida, el calentamiento fue el factor que causó la reducción de actividad en la CBS. En conclusión, la fotosíntesis temprana de la CBS fue gobernada por las precipitaciones ocultas, mientras que el tratamiento de reducción de precipitaciones determinó la duración de la fotosíntesis neta positiva a lo largo del día cuando la lluvia precedente fue relevante.

En la **discusión general**, comentamos las ventajas y desventajas de dos aparatos diseñados e implementados expresamente para esta tesis, así como otros temas metodológicos relacionados con los experimentos. En esta sección enfatizamos los resultados principales contextualizados en su marco teórico, resumidos a continuación:

1. Discutimos las características y funcionamiento de (i) una cubierta móvil para excluir toda la lluvia natural sin causar alteraciones micrometeorológicas significativas en las parcelas experimentales (descrita en el Capítulo I y empleada en los Capítulos I y II); y (ii) una cámara portable para medir el intercambio gaseoso en las CBSs sin extraerlas de sus sitios naturales de crecimiento (descrita en el Capítulo III y empleada en el Capítulo IV). Ambos aparatos han mostrado resultados satisfactorios.
2. Discutimos la relación entre las respuestas ecofisiológicas y las escalas temporales, destacando que: (i) Las respuestas estadísticamente significativas a los factores climáticos cambiaron con la escala temporal. Por ello, la inclusión de distintas escalas de tiempo en este tipo de estudios es esencial para la comprensión de los procesos, y para desentrañar las relaciones de los organismos objeto de estudio con la disponibilidad de agua. (ii) La variabilidad actual de las respuestas ecofisiológicas es clave para evaluar el impacto real del cambio climático. (iii) La memoria ecosistémica a eventos de precipitación anteriores es una característica crítica del funcionamiento los ecosistemas semiáridos, y por ello se debe evaluar la influencia de las características de la lluvia antecedente en las respuestas ecofisiológicas.
3. Establecemos unos umbrales en los que los pulsos de agua desencadenan respuestas ecofisiológicas. En *M. tenacissima*, la estacionalidad y humedad del suelo configurada

por las características de la precipitación antecedente modularon los umbrales de las lluvias efectivas para desencadenar dichas respuestas. Esta especie fue más sensible a los pulsos de agua en verano, promoviendo la respiración y/o fotorespiración cuando las lluvias fueron menores a 5 mm, e incrementando la fotosíntesis neta en eventos mayores. La CBS pueden activar su metabolismo con precipitaciones ocultas menores a 1 mm, pero si su estado de hidratación no se mantiene hasta el comienzo de condiciones lumínicas aptas para la fotosíntesis, la activación de la respiración oscura promueve un balance de carbono negativo.

4. Discutimos las respuestas de los organismos objeto de estudio al cambio climático. *M. tenacissima* usó tanto estrategias de tolerancia como de evitación para enfrentarse a situación de estrés hídrico. Como organismos poikilohídricos que son, las CBSs estuvieron en estado de dormancia en periodos de mayor estrés hídrico. Los dos organismos objeto de estudio pueden resistir grandes niveles de deshidratación y muestran gran resiliencia, por lo que un incremento del periodo de sequía probablemente no afectará a su capacidad de recuperación. Sin embargo, mostraron gran sensibilidad a la frecuencia y duración de los pulsos de agua en los periodos de no sequía, especialmente en invierno, y una reducción de la cantidad de lluvia disminuirá sus tiempos de fijación de carbono.
5. Finalmente discutimos el rol de los ecosistemas limitados por el agua en el ciclo del carbono bajo un escenario de cambio global. Nuestra revisión de la literatura más reciente muestra que: (i) una de las áreas de estudio de esta tesis actúa actualmente como emisor de carbono hacia la atmósfera; (ii) la tendencia de la variabilidad interanual del sumidero de carbono a escala global está controlada por la respuesta de los ecosistemas semiáridos al clima; y (iii) el cambio climático en curso probablemente potenciará un balance neto de emisor de carbono en las áreas semiáridas.

Para concluir el trabajo, al final de esta memoria se ha incluido una sección de **conclusiones generales** donde resaltan las diez ideas fundamentales de la tesis.

JOURNAL CITATION REPORT DE LAS PUBLICACIONES PRESENTADAS

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Ladrón de Guevara, M., Lázaro, R., Quero, J.L., Ochoa, V., Gozalo, B., Berdugo, M., Uclés, O., Escolar, C., Maestre, F.T. (2014). Simulated climate change reduced the capacity of lichen-dominated biocrusts to act as carbon sinks in two semi-arid Mediterranean ecosystems. *Biodiversity and Conservation, 23*, 1787-1807.

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	Q2 in Plant Sciences						
ACTA OECOL	1146-609X	2502	1.617	1.915	0.564	94	7.8
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ANEXO

Copias originales de los capítulos publicados o enviados a revistas científicas

Simulated climate change reduced the capacity of lichen-dominated biocrusts to act as carbon sinks in two semi-arid Mediterranean ecosystems

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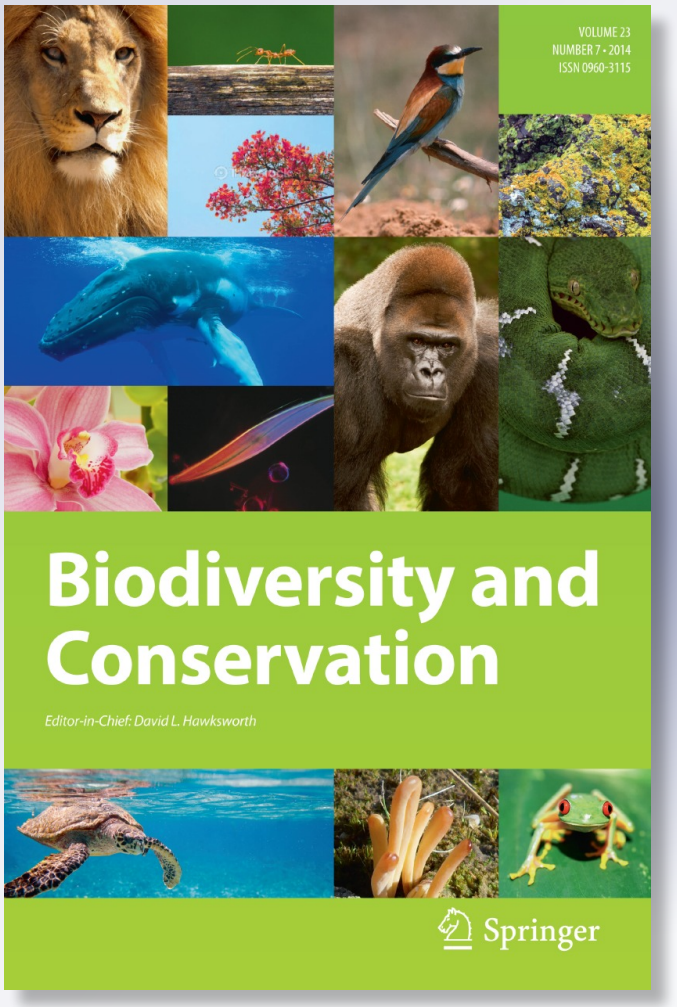

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ORIGINAL PAPER

Simulated climate change reduced the capacity of lichen-dominated biocrusts to act as carbon sinks in two semi-arid Mediterranean ecosystems

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Abstract The importance of biological soil crusts (biocrusts) for the biogeochemistry of drylands is widely recognized. However, there are significant gaps in our knowledge about how climate change will affect these organisms and the processes depending on them. We conducted a manipulative full factorial experiment in two representative dryland ecosystems from central (Aranjuez) and southeastern (Sorbas) Spain to evaluate how precipitation, temperature and biocrust cover affected the assimilation and net C balance of biocrusts. Chlorophyll fluorescence, net photosynthesis and dark respiration were measured in situ bimonthly during a year. We also conducted daily cycle measurements of net photosynthesis in winter and at the end of spring. In Sorbas, warming reduced the fixation of atmospheric C in biocrust-dominated microsites throughout the year. In Aranjuez, there was an interaction between the three factors evaluated; during winter, net photosynthesis was significantly greater in high biocrust cover plots under natural conditions and in the rainfall exclusion treatment. During the daily surveys, rainfall exclusion and warming reduced C fixation in Sorbas and in Aranjuez respectively. The effects of the treatments evaluated varied with the rainfall and non-rainfall water inputs (NRWIs) registered before

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the measurements. Our results suggest that changes in NRWI regimes as consequence of warming could have a greater impact on the C balance of biocrusts than changes in rainfall amounts. They also indicate that climate change may reduce the photosynthetic ability of lichens, with a consequent reduction of their dominance in biocrust communities at the mid to long term. This could reduce the ability of dryland ecosystems to fix atmospheric C.

Keywords Warming · Rain exclusion · Lichens · Gas exchange · Chlorophyll fluorescence · Semi-arid land

Introduction

In drylands, plant interspaces are usually covered by biological soil crusts (hereafter biocrusts), communities composed of bacteria, fungi, algae, lichens, liverworts and mosses (Eldridge and Greene 1994; Belnap 2003; Maestre et al. 2011). The importance of biocrusts for biogeochemical cycles and hydrogeomorphological processes in drylands is widely recognized (reviewed by Belnap 2003; Belnap and Lange 2003; Belnap 2006). While photosynthetic organisms forming biocrusts have lower net photosynthetic rates than vascular plants, in areas where plants are scarce or physiologically limited, biocrusts are considered a major component of the CO_2 uptake by the whole ecosystem (Lange et al. 1992; Garcia-Pichel and Belnap 1996; Lange 2003). Indeed, and at global scale, a carbon (C) net assimilation of $\sim$2.5 Pg a^{-1} by cryptogamic ground covers has been estimated, corresponding to around 4.5 % of net primary production by terrestrial vegetation (Elbert et al. 2012).

Biocrust-forming organisms typically show a fast metabolic response to changes in their microenvironmental conditions, such as water scarcity, high and low temperatures and elevated light exposure (Lange et al. 1997, 1998; Kappen et al. 1998; Kranner et al. 2003; Zheng et al. 2011). Since biocrust constituents occupy the soil surface (usually with higher temperatures than the air during cloudless days), and lack stomatal control, they dry out quickly (Bowling et al. 2011). Hence, they are very sensitive to humidity and temperature pulses, these being the main factors determining their biological activity in drylands (Veste et al. 2001; Grote et al. 2010; Bowling et al. 2011). Most previous studies on the photosynthetic behaviour of biocrust constituents have been conducted in the laboratory (Lange et al. 2001; Lange 2003; Grote et al. 2010), or have separated them from their underlying soil, with the consequent possibility of having eliminated the major factor influencing their CO_2 exchange (Wilske et al. 2008). There are also important uncertainties about how the metabolic activity of biocrusts will be affected by predicted changes in climate, and how climate change-induced effects on biocrusts will impact the C balance of drylands (Maestre et al. 2013). Therefore, more information about the in situ gas exchange of unaltered biocrusts, as well as a better understanding of the relationship between environmental factors and the photosynthetic activity of biocrusts, is necessary to improve our ability to predict how climate change will impact drylands.

Organisms such as lichens may also be suitable model organisms to detect changes in photosynthetic activity as a result of climate change because of their lack of homeostatic mechanisms to cope with extreme environmental conditions (Lechowicz 1982). This fact is important for the new generation of climate models, which consider the feedbacks between vegetation dynamics and climate change (e.g. Randerson et al. 2009; Thornton et al. 2009;

Friedlingstein and Prentice 2010). To improve our understanding on how climate change will impact the physiological activity of biocrusts, we conducted a full factorial climate manipulative experiment at two field sites in Spain. Based on previous studies that highlighted the importance of wetting events in the C balance of poikilohydric organisms (e.g. Čabrajić 2009; Reed et al. 2012), and the negative effects that temperature increases have on maintaining soil moisture (e.g. Grote et al. 2010; Su et al. 2012), we hypothesized that predicted changes in climate will have a negative effect on the ability of biocrusts to fix carbon (Maestre et al. 2013), and thus in the capacity of dryland ecosystems to be C sinks. Our objectives were to: (i) explore the main temporal features of the biocrust C fixation ability on daily and seasonal scale, and how they relate to climatic variables; (ii) evaluate how predicted changes in climate (warming of 2–3 °C and ~30 % reduction in rainfall; Escolar et al. 2012) will affect the in situ photosynthetic efficiency and gas exchange of biocrusts; and (iii) verify whether climate change effects on these variables vary with the degree of biocrust development.

Methods

Study sites

The study was conducted at two research sites: Aranjuez (40°02′N–3°32′W; 590 m a.s.l.) and Sorbas (37°05′N–2° 04′W; 397 m a.s.l.), located in central and south-eastern Spain, respectively. Their climate is Mediterranean semi-arid, with a mean annual temperature and rainfall of 15 °C and 349 mm, and 17 °C and 274 mm in Aranjuez and Sorbas respectively. Both sites are Miocene gypsum outcrops, with soils classified as and are classified as Gypsiric Leptosols (IUSS Working Group WRB 2006). The cover of perennial vegetation is below 40 % at both study sites, and is constituted mainly by the tussock grass *Macrochloa tenacissima* (=*Stipa tenacissima*, alpha grass). Some shrubs or dwarf shrubs (e.g. *Retama sphaerocarpa, Gypsophyla struthium, Helianthemun squamatum*), as well as some crass perennial plants (e.g. *Sedum gypsicola, Sedum sediforme*), are also common to both areas. Over half of the plant interspaces are colonized by a well-developed biocrusts dominated by green algae lichens in both sites, being the most abundant *Diploschistes diacapsis*, *Squamarina lentigera, Cladonia convoluta, Fulgensia spp.* and *Psora decipiens* (see Maestre et al. 2013 for a species checklist).

Experimental design

A full factorial manipulative experiment was setup in July 2008 and May 2010 in Aranjuez and Sorbas, respectively. The factors, all of them with two levels, were: (i) biocrusts cover (Crust; poorly developed biocrusts communities with cover <25 % vs. well developed biocrusts communities with cover >50 %); (ii) warming (WA; natural vs. 2–3 °C temperature increase); (ii) and rainfall exclusion (RE; natural vs. ~30 % rainfall exclusion). Throughout the text we refer to the control treatment as the natural temperature and rainfall conditions. The number of replicates was 10 and 8 in Aranjuez and Sorbas, resulting in a total of 80 and 64 experimental plots at these sites, respectively. A minimum separation distance between plots of 1 m was established to minimize the risk of sampling non-independent areas. The plots were 1 m^2 with a PVC collar of 20 cm in diameter in their centers for biocrust monitoring. The collars were inserted approximately 5–6 cm into the soil, standing out 2–3 cm over it.

The warming treatment aimed to simulate the average of predictions derived from six Atmosphere–Ocean General Circulation Models for the second half of the 21st century (2040–2070) in central and Southeast Spain, which predict increases of 2.1–2.3 °C during winter months, and 3.2–3.5 °C during summer months (De Castro et al. 2005). To achieve a temperature increase within this range, we used open top chambers (OTCs) of hexagonal design with sloping sides of 40 × 50 × 32 cm (see Escolar et al. 2012 for details). These structures were built with methacrylate, and were elevated between 3 and 8 cm from the soil to allow air circulation over the surface of the plots, and thus avoid an excessive overheating.

Predicted changes in rainfall for our study area are subjected to a high degree of uncertainty, but most climate models forecast important reductions—between 10 and 50 %—in the amount of spring and fall rainfall (De Castro et al. 2005). To simulate these conditions, we set up passive rainout shelters (RSs) based upon the design described by Yahdjian and Sala (2002). Each RS has an area of 1.44 m^2 (1.2 × 1.2 m), a mean height of 1 m, and is composed of three methacrylate grooves, which cover approximately 37 % of the surface, connected to plastic bottles that accumulate the excluded water in order to control it. See Escolar et al. (2012) and Maestre et al. (2013) for additional details of the experimental installations and their effects on microclimatic variables.

Microclimate monitoring

The effects of the OTCs and RSs on air temperature and relative humidity (RH) were continuously monitored using automated sensors (HOBO U23 Pro v.2 Temp/RH, Onset Corporation, Bourne, MA, USA), and those on soil surface (0–2 cm) temperature and moisture (0–5 cm depth) were continuously monitored on the two levels of Crust factor using TMC20-HD and EC-5 sensors, respectively (Onset Corp. and Decagon, Inc., Pullman, WA, USA). Soil moisture (0–5 cm depth) was measured discontinuously with time-domain reflectometers (TDR, Topp and Davis 1985). To compare these effects between treatments, air and soil temperatures were calculated as the temperature average during the gas exchange measurement period, and soil moisture measured discontinuously was preferably used.

Two sources of non-rainfall water input (NRWI) were studied: dew and fog. Several studies have found a positive and significant relationship between the duration of these events and the amount of water condensed on the surface (e.g. Zangvil 1996; Beysens et al. 2005; Kidron 2000; Uclés et al. 2013a). But the main advantage of detecting these events lies in providing information on the duration of their occurrence, which could even be more important than their amount regarding biological activity and species distribution (Malek et al. 1999; Kidron and Temina 2013). Hence, dew and fog condensations were studied in terms of their durations, and the NRWI between plots with and without OTC was compared based on this assumption.

Dew occurs when the temperature of the surface where water condenses is equal or lower than the dew point of the surrounding air. Measuring the temperature of the soil surface with the enough accuracy to determinate dew with in situ sensors is difficult, but previous studies have done so using thermocouples buried in the soil (Uclés et al. 2013a, b). Hence, we estimated the duration of dew as the time in which the surface temperatures of the sensors TMC20-HD were below the dew point temperature of the air (this being calculated from the temperature and RH measured by the HOBO U23 sensors).

Fog is produced when the atmospheric water vapour concentration reaches saturation and condensed water droplets remain suspended in the air. In this study, the occurrence of

fog events was considered when relative air humidity was higher than 98.5 %, as the sensors typically have an accuracy error of ±2.5 % (Onset Corporation 2013).

Gas exchange measurements

The CO_2 exchange of the whole soil column, including the biocrusts living on its surface, was measured at six randomly chosen replicates per combination of treatments every 2 months between September 2010 and January 2012. These measurements were conducted at both study sites after dawn, starting when the collars received direct light, and always on cloudless days to avoid the interference of occasional clouds in the response of any treatment, or hour in the daily cycles. This is a representative meteorological situation of the experimental areas as, on average, Aranjuez and Sorbas have 94 and 106 cloudless days per year, respectively; AEMET 2013). Previous studies have found that, in drylands with relevant water supply as dew or fog, such as those we studied, the photosynthetic activity of biocrust constituents peaks during early morning hours in the absence of rain (Kappen 1988; Lange et al. 1992, 2006; Veste et al. 2001). In addition to the absence of clouds, we performed all measurements within a maximum interval of 2 h to maintain homogeneous environmental conditions, so half of the replicates were measured in 1 day, and the other half were measured on the next day.

We measured net photosynthesis (μmol CO_2 $m^{-2}s^{-1}$) using two infrared gas analyzer (IRGA) systems (LI-6400, Lincoln, NE, USA) connected to a customized methacrylate transparent chamber with a volume of 2,385 cm^3. To ensure the sealing of the chamber on the sample, a toroidal flat disk covered with an air-tight rubberized band was placed between the chamber and the PVC collar. The devices were operated as open dynamic systems, i.e., the chamber air was renewed with a continuous external air flow and the CO_2 flux was calculated as the difference in the [CO_2] between the chamber air (measured by an IRGA referred to as sample) and the external input air (measured by an IRGA referred to as reference). The external input air was taken at two meters above the soil surface by a rod to use a natural atmospheric air without the influence of the biological activity (photosynthesis and respiration of soil and plants) and the operator's breathing on its [CO_2], and a bottle of 6 L was used as a buffer volume in order to use an input air with a stable [CO_2]. The external air circulated through the system with a flow of 800 μmol s^{-1}, and an additional ventilation into the chamber of 0.7 m s^{-1} were used to obtain an adequate air mixing. System flow and IRGA CO_2 zeros were calibrated each day before measurements, and H_2O zeros were calibrated the first of the two consecutive measurement days of each bimonthly data collection. Before each individual measurement we matched the CO_2 readings of the sample and reference IRGAs, as this is very important for the accuracy of the measurements when low ΔCO_2 values are expected (LI-COR 2012). The net photosynthesis value of a plot was recorded when the CO_2 values of the sample and reference IRGAs were stabilized.

Photosynthetically active radiation (PAR) was measured with a LICOR external quantum sensor (9901-013, LICOR, Lincoln, NE, USA), and with a Field Scout Quantum Light Meter (Spectrum Technologies, Plainfield, IL, USA), both of them placed at the top of the transparent chamber to have a reference value close to the biocrust surface. It was recorded at the same time of the net photosynthetic activity, and was used to consider in the statistical analyzes a potential source of variability in photosynthesis values because of environmental changes unrelated to our treatment. Immediately after these records, dark respiration was measured in the same plots with two soil respirometers (LI-8100, Lincoln, NE, USA). They operated as closed dynamic systems, i.e., there was a continuous

recirculation of air inside the system, but without being renewed with an outside air intake. CO_2 flux was calculated as the change in chamber [CO_2] during the sample enclosure time adjusted to an exponential function. Each respiration measurement lasted 120 s, this being the recommended time when is expected the low flux characteristic of dryland ecosystems (Castillo-Monroy et al. 2011; Rey et al. 2011). A fitting equation between these two systems (LI-6400 and LI-8100) was performed covering the clear chamber attached to LI-6400 with an opaque cloth ($R^2 = 0.96$), and it was applied on the dark respiration records. As both devices measured gas exchange of the whole soil profile, in both cases the respiration of autotrophic and heterotrophic components was present, and gross photosynthesis could be calculated as the sum of net photosynthesis and soil dark respiration.

Daily cycles of net photosynthesis were also conducted in May 2010 in Aranjuez, and in January 2012 at both study sites. They were performed with the same protocol described above, but the measurements were extended throughout the daylight, and only collars with well-developed biocrusts were measured. During the measurements conducted in May 2010 only net photosynthesis was measured, but in January 2012 net photosynthesis (Li-6400) and dark respiration (LI-8100) were taken, so we could estimate gross photosynthesis.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured by computing the parameter F_v/F_m, an indicator of the status of the photosystem II in photosynthetic organisms (Maxwell and Johnson 2000), with a Handy PEA fluorometer (Hansatech instruments, Kings Lynn, UK). Low values indicate the presence of physiological stress (Maxwell and Johnson 2000). These measurements were conducted simultaneously with the bimonthly gas exchange measurements described above, but only in the Sorbas site. F_v/F_m was calculated as the ratio between the variable ($F_v = F_m - F_o$) and the maximum (F_m) fluorescence signal. Prior to measurements, lichens were dark adapted using opaque cloths for 30 min. Fluorometer measurements of the whole biocrusts community were made in four replicated plots per combination of treatments. Six random measurements per plot were taken in all cases, which were averaged for further analyses.

Data analyses

We evaluated the effects of biocrust cover (Crust), warming (WA), rain exclusion (RE), and their interactions, on the measured variables (net and gross photosynthesis, dark respiration and F_v/F_m) over time using general linear mixed models (LMMs) by means linear mixed-effects models (lme) procedure from Linear and Nonlinear Mixed Effects Models (nlme) R package (Pinheiro et al. 2013). The generic function of lme fits a linear mixed-effects model in the formulation described in Laird and Ware (1982), but besides allowing for nested random effects (Pinheiro et al. 2013). LMMs expand on the ordinary linear regression model by allowing one to incorporate lack of independence between observations and to model more than a single error term (Cnaan et al. 1997), so temporal correlated errors and variance of the errors can be modeled in order to avoid pseudoreplication and heteroscedasticity problems, respectively.

Four fixed factors were established in the bimonthly survey analyses: measurement date, Crust, WA and RE. All their possible interactions were included in the models. In daily cycle analyses, the Crust factor was not applied, as only high biocrust cover plots (>50 %) were measured. The analyses were implemented in InfoStat software version 2013p (Di

Rienzo et al. 2013), a friendly interface to use R (version 2.15.2). The restricted maximum likelihood (REML) procedure, which takes into account the loss in degrees of freedom resulting from estimating fixed effects (Patterson and Thompson 1971), was used to estimate the variance and covariance components. The best structures for our models were selected with the AIC criterion (Akaike 1973). For the analyses of net and gross photosynthesis, we also evaluated whether the inclusion of PAR as a covariate in the models improved their fit. Fisher's least significant difference (LSD) post hoc test was applied on the most complex interactions of the factors that showed significant differences. The effect of WA on NRWI duration was analyzed for each measurement day using *t* tests. When the variance was not homogeneous, we used the non parametric Mann–Whitney test instead.

Results

Treatment effects on micrometeorological conditions during measurements

Summer drought was more acute in Sorbas than in Aranjuez (Fig. 1). The distribution of rainfall events was also less equitable in Sorbas site. However, the amount of rainfall registered in the days prior to the measurements was greater in Sorbas, especially in December 2010, September 2011, and November 2011. The winter 2011–2012 was characterized by an unusually low number of rainfall events in Aranjuez. Nighttimes preceeding the measurements were characterized by the formation of dew and fog in Aranjuez and Sorbas, particularly during winter. Dew formation and small events of fog predominated in Sorbas, whereas long duration fog events (more than 4 h) were more common in Aranjuez. Warming reduced the duration of dew and fog at all events, albeit significant differences were only found in short events (two in Sorbas, and another two in Aranjuez; Fig. 1).

During the bimonthly measurements, the WA+RE treatment had consistently the lowest soil moisture values in Sorbas (Fig. S1a, b), whereas in Aranjuez the treatment with such values varied through time and with the degree of biocrust cover (Fig. S1c, d). OTCs caused a soil temperature increase of 1.7 and 3.3 °C on average in Sorbas and Aranjuez respectively (Fig. S2a, b). In Sorbas, this treatment also increased early morning air temperature 2.4 °C in February and 5.6 °C in April (Fig. S2c). In Aranjuez (Fig. S2d), this increase was over 2 °C only in February (2.4 °C), and was lower or did not occur in the rest of dates at both sites, as the temperature differences in plots with and without OTCs were between -1.8 and 1.6 °C. During the January daily cycles (Fig. 2a, b), OTCs caused a maximal increase in air and soil temperatures of 4 and 3.3 °C, respectively, in Sorbas, and of 4.3 and 3.5 °C in Aranjuez. In May (Fig. 2c), the maximal increase in air temperature in Aranjuez was greater (6.8 °C), but increases in soil temperature were similar to those recorded in winter (3.3 °C).

Temporal features of the biocrust gas exchange on seasonal and daily scales

Positive net photosynthesis was only detected during late autumn and winter in both Aranjuez and Sorbas (Fig. 3), coinciding with the peak of gross photosynthesis (Fig. 4). The maximum peaks of gross photosynthesis were observed in the high biocrust cover plots at both study sites (Fig. 4). In Sorbas, dark respiration peaks occurred in late spring in low biocrust cover plots under the RE, WA and RE+WA treatments, and in summer in the rest of the treatments. In Aranjuez, these peaks were detected in spring in low biocrust

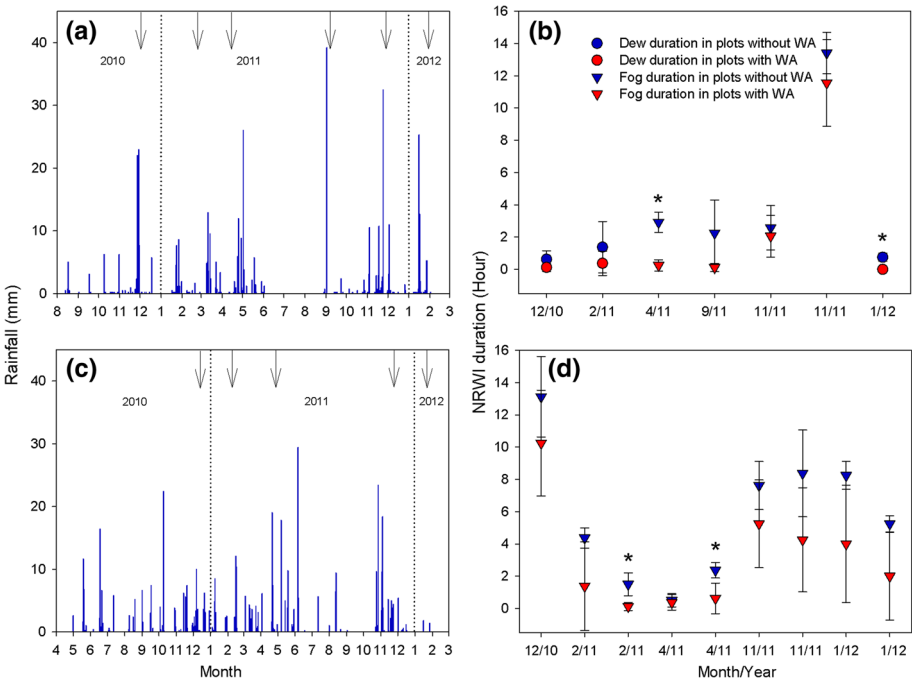

Fig. 1 Rainfall registered during the study period at Sorbas (**a**) and Aranjuez (**c**), and non-rainfall water inputs (NRWI) registered before the gas exchange and chlorophyll fluorescence measurements at Sorbas (**b**) and Aranjuez (**d**). *Asterisks* denote statistically significant reduction of NRWI at $p < 0.05$ in warming treatment (WA) plots. NRWI data are mean ± SE ($n = 4$)

cover plots under the control, RE and WA treatments, and in the rest of the treatments during late autumn and winter.

Seasonal effects on net photosynthesis were evident in Aranjuez. Antecedent rainfall inputs between December 2010 and April 2011 were similar, and the differences in other environmental variables (e.g., air and soil temperatures, soil moisture) were not relevant during early morning hours at this site (Figs. S1c, d, S2b, d). The most relevant difference between these two months was the NRWI, which was greater in December (Fig. 1), but presumably its effect on photosynthesis was not significant, since precedent rainfalls were abundant. However, important contrasts in the response of the biocrusts were observed, with maximum annual net photosynthesis values in December, and negative values for all treatments in April (Fig. 3c, d); so the biocrust gas exchange response to similar environmental conditions was different depending on the season.

Due to the random nature of rain events in drylands, the photosynthetic activity can also show a high intra-seasonal variability. For example, the effect of the previous winter rainfalls on in situ net photosynthesis over biocrust-forming lichens can be observed comparing December 2010 with February 2011. The C fixation differences between these months were remarkable in both sites because preceding precipitation was much more important in the fist than in the second month (Fig. 1).

The January daily cycles obtained at both sites were very different due to the distinct water availability of the specific measured days (Fig. 1, see antecedent rainfalls). This caused differences in gross photosynthesis; as this variable was around three-fold higher in

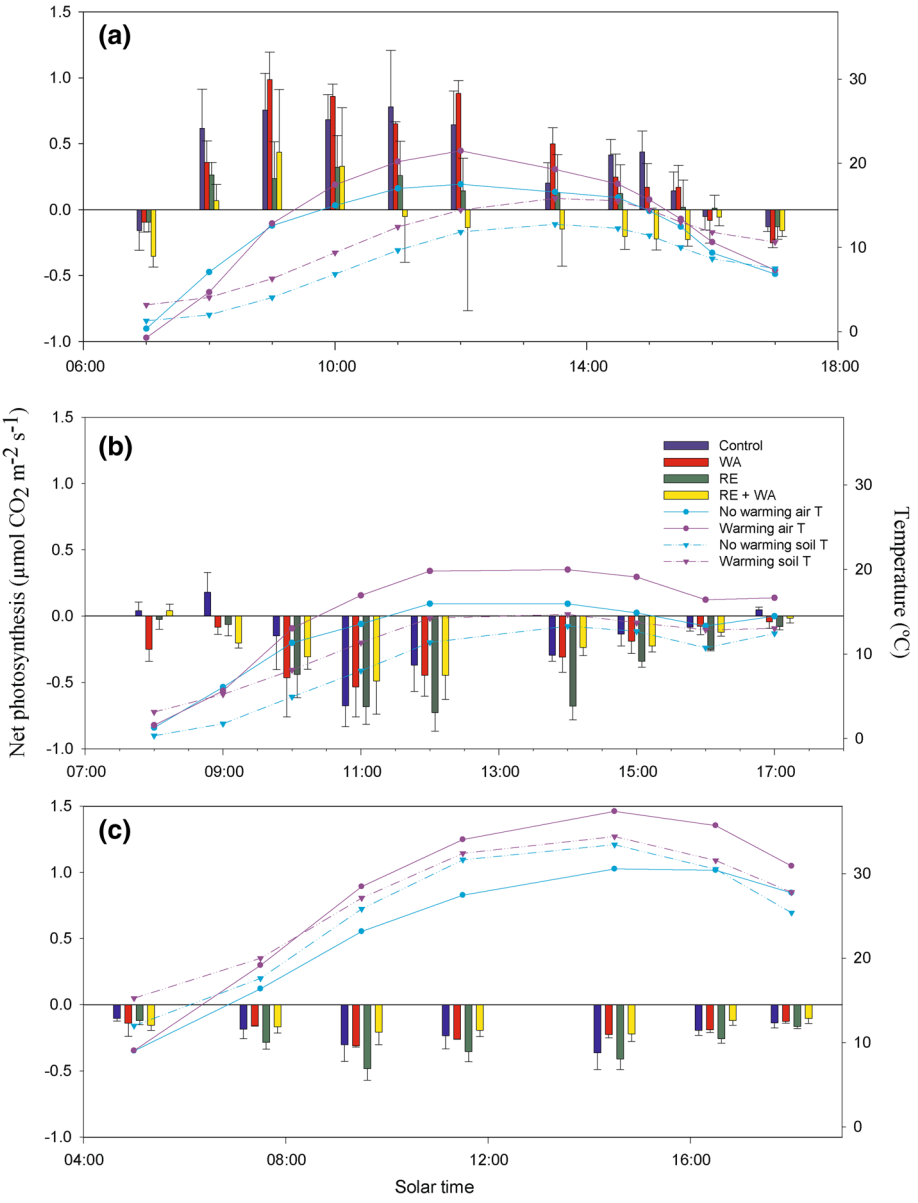

Fig. 2 Daily cycle surveys of net photosynthetic rate in January at Sorbas (**a**) and Aranjuez (**b**), and in May at Aranjuez (**c**). Data are mean ± SE ($n = 6$ in January; $n = 4$ in May). *WA* warming, *RE* rainfall exclusion

Sorbas than in Aranjuez (Fig. 5). At Sorbas (Fig. 2a), net photosynthesis remained positive in all plots (excepting those under the RE+WA treatment) when PAR values were higher than 150 µmol m^{-2} s^{-1}, and a maximum peak was detected at noon. In Aranjuez (Fig. 2b), positive net photosynthesis was only recorded at dawn and dusk in two treatments (control and RE+WA). During the May daily cycle in Aranjuez (Fig. 2c), no positive values of net photosynthesis were observed at any time.

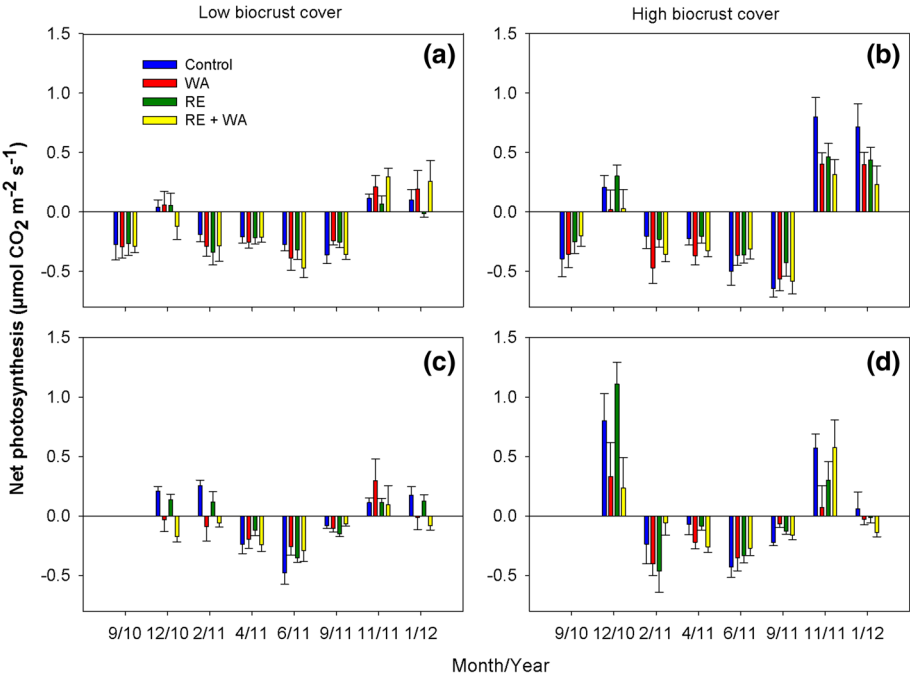

Fig. 3 Temporal variation of net photosynthetic rate at Sorbas (**a**, **b**) and Aranjuez (**c**, **d**) experimental sites. Data are mean ± SE ($n = 6$). *WA* warming, *RE* rainfall exclusion

Impacts of warming and rainfall reduction on the in situ photosynthetic efficiency and gas exchange of the biocrusts

Variation of the effects of climate change as a function of biocrust cover

In the analysis of the bimonthly surveys, where the factor Crust was included, rainfall exclusion and temperature increase showed complex interactions with biocrust cover and the time of measurement, which affected all the variables measured in Sorbas, and the net photosynthesis in Aranjuez. Warming had a greater effect on biocrust performance at Sorbas than at Aranjuez, and reduced C fixation in areas with well-developed biocrusts. Significant interactions found with the statistical models are disclosed below in detail.

A significant Date × Crust × WA interaction was found when analyzing F_v/F_m data from Sorbas (Table S1). An analysis of the Crust × WA interaction for the different surveys revealed that, in December 2010, plots with low biocrust cover and without OTCs had significantly lower F_v/F_m values than those with high biocrust cover plots (regardless of the presence of OTCs). In November 2011, low biocrust cover plots had significantly lower F_v/F_m values than high biocrust cover plots, regardless of the WA levels considered. No significant differences were found in the factor RE (Fig. 6d).

When analyzing net photosynthesis data, a significant Date × Crust × WA interaction was also found at Sorbas (Table S2). When this variable was positive, WA reduced it in high biocrust cover plots (Fig. 6a). But in low biocrust cover plots, net photosynthesis was increased with WA during the last measured winter month. High biocrust cover plots had greater positive C balance than low biocrust cover plots without OTCs, but these

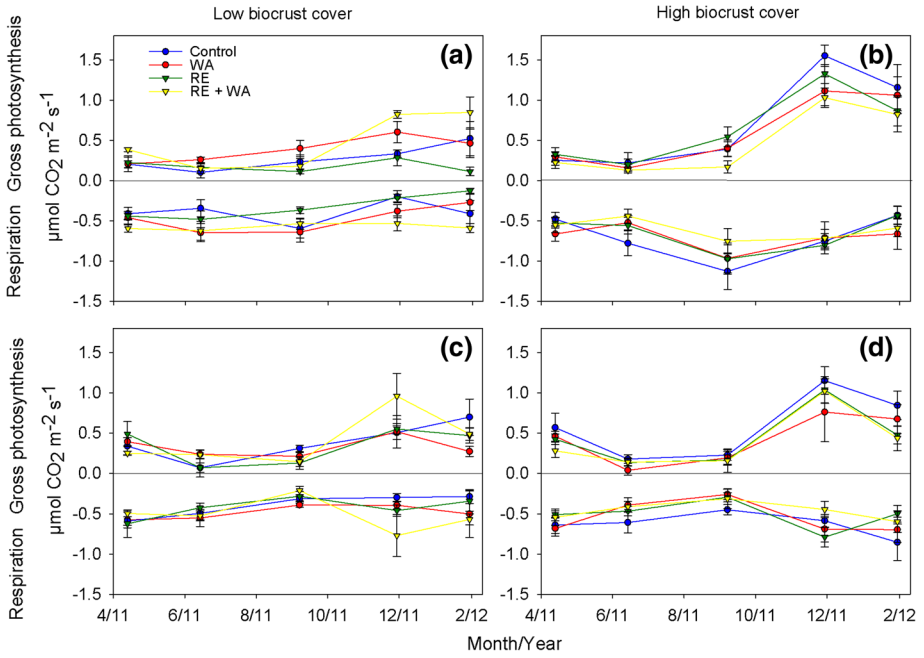

Fig. 4 Temporal variation of gross photosynthetic rate and dark respiration at Sorbas (**a**, **b**) and Aranjuez (**c**, **d**) experimental sites. Data are mean ± SE ($n = 6$). *WA* warming, *RE* rainfall exclusion

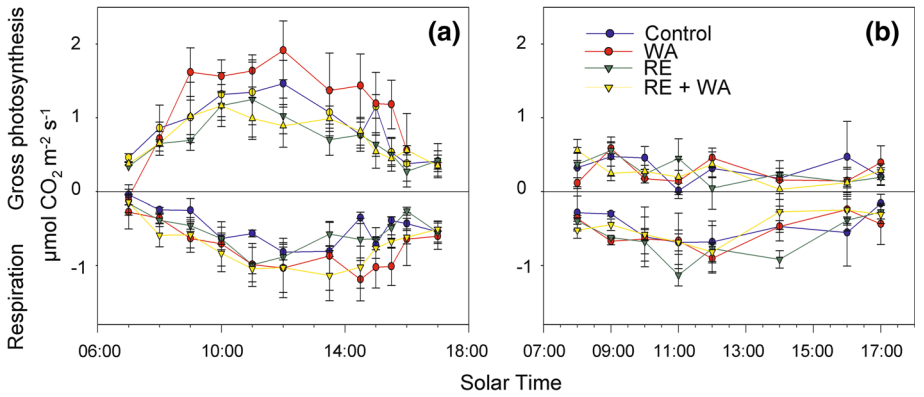

Fig. 5 Daily cycle surveys of gross photosynthetic rate and dark respiration in January at Sorbas (**a**) and Aranjuez (**b**) experimental sites. Data are mean ± SE ($n = 6$). *WA* warming, *RE* rainfall exclusion

differences were not found in the WA treatments. In February and April 2011, when net photosynthesis was negative, WA increased the C emissions in high biocrust cover plots, an effect that was not found in the low biocrust cover plots. No significant effects of RE were found in Sorbas, but in Aranjuez a significant Date × Crust × WA × RE interaction was found (Table S2). In December 2010, control and RE plots with high biocrust cover had a significantly higher net photosynthesis than those from other treatments (Fig. 7a). However, only low biocrust cover plots with control and RE treatments had a positive net

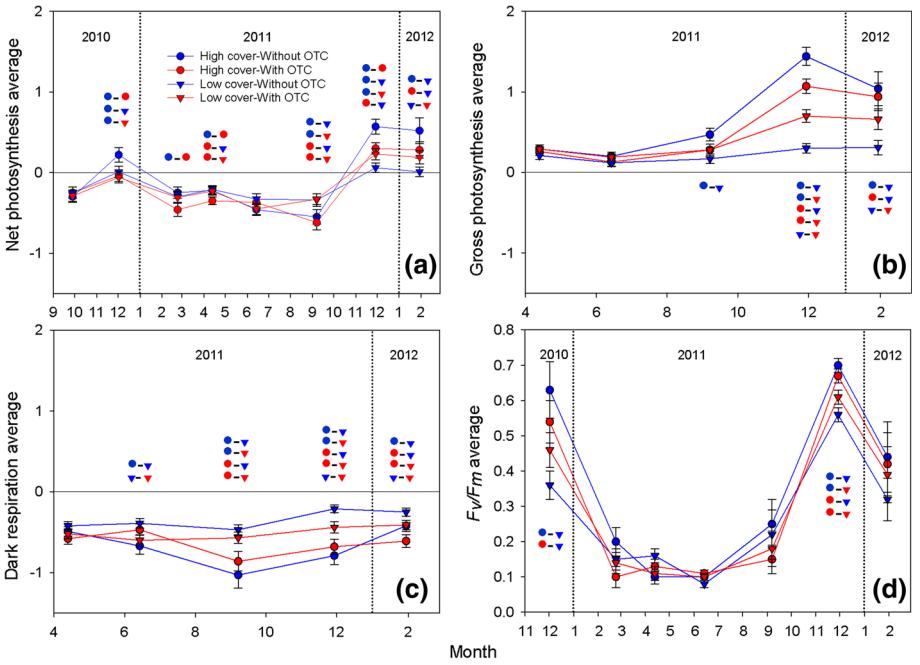

Fig. 6 Fisher's least significant difference (LSD) post hoc results in Sorbas bimonthly surveys: net photosynthetic rate (**a**), gross photosynthetic rate (**b**), dark respiration (**c**) and F_v/F_m (**d**). Treatment *symbols* joined by *dashes* denote statistically different averages at $p < 0.05$ between these treatments within each time. Data are mean ± SE ($n = 6$). *WA* warming

photosynthesis in February 2011 (Fig. 7b). At this date, the values found in these plots were significantly greater from those found in high biocrust cover plots with either WA or RE, but not with WA+RE treatments.

A significant Date × Crust × WA interaction was found when analyzing gross photosynthesis data from Sorbas (Table S2). Only low biocrust cover plots had significant differences between WA factor levels, as this variable was higher in WA plots during late autumn and winter. In plots without OTCs, gross photosynthesis was significantly higher in high biocrust cover plots than in low biocrust cover plots during September 2011, November 2011 and January 2012 (Fig. 6b). In WA plots, this difference was only found in November 2011. In Aranjuez, a significant Date × Crust interaction was found; high biocrust cover plots had significantly higher values of gross photosynthesis than low biocrust cover plots in late autumn, when maximum photosynthetic rates were reached (Fig. 7c). Rainfall exclusion had not significant effects on gross photosynthesis.

A Date × Crust × WA interaction close to statistical significance was found when analyzing dark respiration in Sorbas (Table S2). As when F values exceed 2 there is a high probability that the results would have been significant with more replicates (Fry et al. 2013), a post hoc analysis for this interaction was performed. Only low biocrust cover plots showed significant differences between WA levels; higher dark respiration was observed in WA plots in late spring (Fig. 6c). Differences between Crust factor levels were also observed: in plots without OTC high biocrust cover showed higher dark respiration than low biocrust cover in four months (June 2011, September 2011, November 2011 and January 2012); this was also found in plots with OTC in September 2011 and January

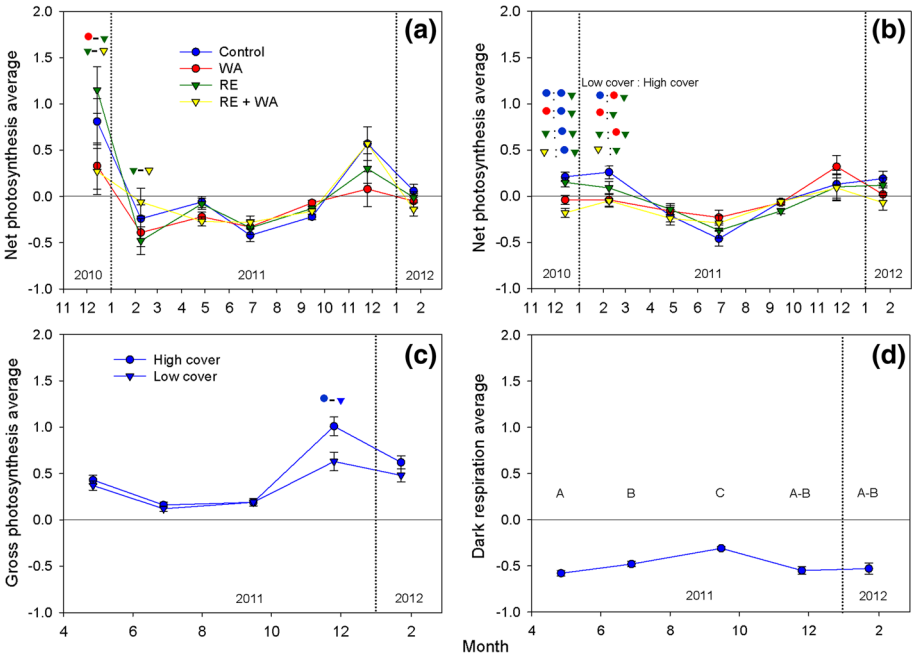

Fig. 7 Fisher's least significant difference (LSD) post hoc results in Aranjuez bimonthly surveys: net photosynthetic rate in high biocrust cover plots (**a**), net photosynthetic rate in low biocrust cover plots (**b**), gross photosynthetic rate (**c**) and dark respiration (**d**). In panels (**a**) and (**c**), the pairs of treatments with statistically different averages within each time are denoted by their legend symbols joined by *dashes*. In panel (**b**), there are no significant differences between the treatments represented within each time. However, significant differences between biocrust cover treatments (i.e. statistical comparison between panels (**a**, **b**)) within each time are shown above the averages. The *first column of symbols* represents the low cover treatments, and after colons, the high cover treatments where significant differences were found. In panel (**d**), significant differences between surveys are shown by *letters*. No *letters* in common denote different averages. All analyses were performed at $p < 0.05$. Data are mean ± SE ($n = 6$).*WA* warming, *RE* rainfall exclusion

2012. In Aranjuez, Date significantly affected dark respiration, with the highest monthly average found in April, (Fig. 7d).

Variation of the effects of climate change as a function of the time scale and water availability

The effects of our treatments also differed with the time scale considered (bimonthly or daily measurements), and with the characteristics of the preceding rainfall and NRWI events. For example, although warming was the most important factor determining responses in Sorbas at early morning hours during bimonthly measurements, rainfall exclusion was the main factor limiting net photosynthesis during the winter daily cycle at this site, as a significant Time × RE interaction was found (Table S3). Plots with rainfall exclusion had significantly lower net photosynthesis than plots without it from 10 A.M. to 2 P.M. (Fig. 8a). So natural precipitation maintained soil moisture at suitable levels allowing photosynthetic activity throughout the day, something that was not achieved when rainfall was experimentally reduced.

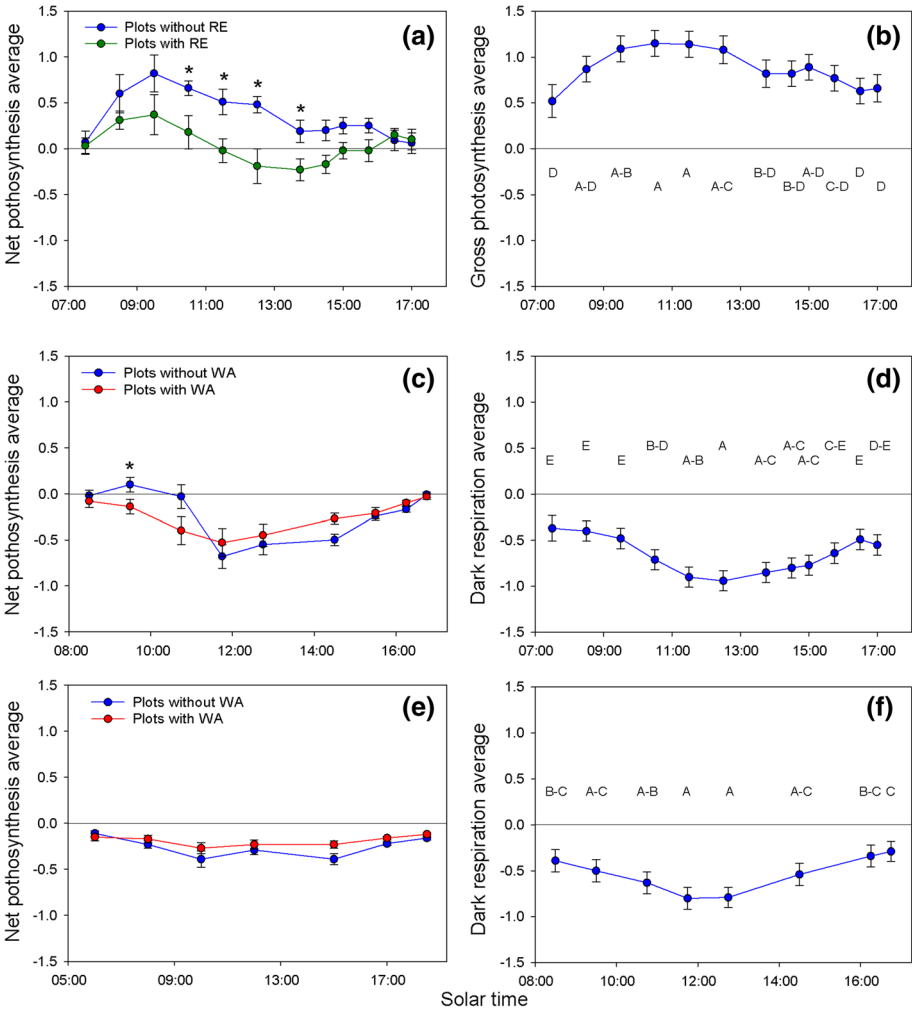

Fig. 8 Fisher's least significant difference (LSD) post hoc results from daily cycle surveys: net photosynthetic rate (**a**), gross photosynthetic rate (**d**) and dark respiration (**e**) in January at Sorbas; net photosynthetic rate (**b**) and dark respiration (**f**) in January at Aranjuez; and net photosynthetic rate (**c**) in May at Aranjuez. In panels (**a**–**c**), statistical differences between the two treatments within each time are denoted by asterisks. In panels (**d**–**f**), significant differences between surveys are shown by letter intervals (e.g., a label *A-C* means that a time contains the letters *A*, *B* and *C*). No *letters* in common denote different averages. All analyses were performed at $p < 0.05$. Data are mean ± SE ($n = 6$). *WA* warming, *RE* rainfall exclusion

In Aranjuez, where rainfalls preceding the winter daily cycle measurements were much lower than in Sorbas (Fig. 1), a significant time × WA interaction was found in both January and May (Tables S3 and S4). In January, net assimilation rate was higher and positive in plots without OTC, and negative in plots with OTC, but only from 9 A.M. to 10 A.M (Fig. 8b). In May, the post hoc test did not show significant differences between plots with and without OTC measured in the same hour (Fig. 8c).

Regarding the other gas exchange variables, significantly higher gross photosynthesis values were found in the morning than in the afternoon in Sorbas (Fig. 8d), while none of the factors evaluated affected gross photosynthesis in Aranjuez (Table S3). The dark respiration values found were similar in Aranjuez and Sorbas, and at both sites peaked at midday(Fig. 8e, f; Table S3).

Discussion

The importance of water availability on biocrust activity

In agreement with previous studies (Lange and Green 2003; Wilske et al. 2008), our results show that seasonal variations in the physiological status and the CO_2 exchange of biocrust-dominated microsites are strongly influenced by the hydration state of biocrust constituents. The maximum photochemical efficiency of PSII (F_v/F_m) reflected the typical physiological activity of biocrusts in the Mediterranean, characterized by a strong seasonal oscillation, with a peak of activity in winter, the rainy season of this climate, and a phase of dormancy in summer, the dry season (Wilske et al. 2008; Pintado et al. 2010). The drastic reduction of F_v/F_m observed during spring and summer (Fig. 6d) indicates a photoinhibition situation resulting from damage to PSII reaction centers, or a down-regulation of PSII (Calatayud et al. 1997). We favour the predominance of the second explanation, as when biocrusts returned to better moisture conditions they recovered the maximum F_v/F_m values, and had similar photosynthetic rates to those observed before the dry season. However, biocrust did not reach an optimal photochemical efficiency at our study sites until October, as was shown by Pintado et al. (2010), who monitored in situ chlorophyll fluorescence of biocrust-forming lichens during several years in a site near to Sorbas. This can be seen with the September 2011 measurements in Sorbas, which were conducted after a relevant rain, and with soil temperature values suitable for the activity of biocrusts. In spite of these conditions, respiration was greater than photosynthesis, generating the most negative C balance (C source) found at this site.

Hydration status also had an important role in the metabolic activity of biocrusts over the day. The differences observed in the January daily cycle between Aranjuez and Sorbas can be explained by the unequal rainfall conditions among them. The major water inputs, and consequently higher soil water content found in Sorbas during this month, allowed optimal conditions for C fixation along the day (Fig. 2a), whereas in Aranjuez the lower rainfalls only allowed positive C fixation at early and late hours (Fig. 2b). In May 2010, and while ten days prior to the measurements a rain event of $\sim$10 mm took place in Aranjuez, the net photosynthesis was negative throughout the day (Fig. 2c), probably because an important part of the water supply had already evaporated, and also because at that time of the year the photosynthetic capacity of lichens is lower than in winter.

Water pulses and degree of biocrust development as modulators of its responses to climate change

The occurrence, magnitude and timing of the two NRWI sources considered (dew and fog) are key to interpret the results and the differences found between Sorbas and Aranjuez during the bimonthly surveys. The RE treatment reduced the amount of rainfall reaching the plots, but did not affect the water inputs coming from NRWI. Nocturnal and early morning moistening through dew and fog enables photosynthesis immediately after

sunrise, which ceases when biocrusts dry out (Kappen 1988), after ca. 2 h. However, high air temperature accelerates the process of thallus dehydratation, a major environmental stressor in lichens (Hájek et al. 2006). Given all this, the main effects of the warming treatment in our experiment were: (1) the reduction of the early morning wetting time on the thalli, by decreasing the formation of dew on the surface of the biocrusts and intercepting fog; and (2) the reduction of the soil water content because the warming increased evaporation. Therefore the negative effect of WA treatment on biocrust photosynthetic performance was due to its reduction of water availability, also in agreement with Maphangwa et al. (2012). Direct effects of warming on the photosynthetic efficiency of biocrusts can be discarded, as the early morning temperature ranges in plots with and without OTC (Fig. S2) were within the photosynthetic optimal of the dominant lichens from our study sites (Lange et al. 1997, 1998; Lange and Green 2004). Johnson et al. (2012) and Zelikova et al. (2012) also found very limited effects of a 2–3 °C warming alone on biocrust communities dominated by cyanobacteria, cyanolichens and mosses.

Warming had a greater effect on gas exchange variables in Sorbas than in Aranjuez in the bimonthly survey, and this was likely due to the differences in NRWI magnitude observed among the study sites. In Sorbas, dew is the most frequent water input. For example, in a study conducted near this site, dewfall condensation and rain/fog inputs were observed during 78 and 16 % of the nights, respectively (Uclés et al. 2013a). However, fog is the main NRWI source in Aranjuez, as it occurred during 27 % of the nights of this study period, while rainfalls only occurred during 8 % of the nights. In all measured winter months, there was dew formation at Sorbas, and fog events were encountered at Aranjuez (Fig. 1). Since dew is directly related to the temperature of the soil surface, warming reduced dew condensation. Our OTCs were also a barrier to the entry of the fog into the plots, but as they were open at the top and elevated from the soil, only reduced significantly the duration of the small fog events. This effect on small NRWI events probably is critical for early net C balance, since they may be at the limit of providing enough water to compensate and surpass respiration activity. So, as warming had a greater effect on small NRWI events, which were more frequent in Sorbas, this could be the main explanation of why the effects of WA factor were most significant in Sorbas than in Aranjuez when analyzing bimonthly data.

Contrary to what we expected, the rainfall exclusion did not have a detrimental effect at Sorbas in the bimonthly surveys, and even this treatment had higher net photosynthesis than in plots with WA and WA+RE treatments (Fig. 7a) at Aranjuez in December 2010. This fact may also be explained because of the importance of the antecedent NWRIs. During the nighttime previous to the measurements, there were 13 h of fog at Aranjuez, and the rainfall shelters did not reduce the amount of fog as the OTCs did (Fig. 1). Therefore, these results could indicate a more important modulation of the early photosynthesis by NRWI pulses than by rainfalls along an annual cycle. When preceding precipitation was abundant, rainfall exclusion had an effect on the evolution of gas exchange during the whole day, as in the case of Sorbas (Fig. 8a). However, when rainfalls were not large enough to maintain a positive C balance during the whole day, as occurred in the Aranjuez winter daily cycle, temperature became the main factor driving biocrust activity (Fig. 8b). The observed net photosynthesis in control plots during early morning at this site was probably activated by nocturnal fog events, and the WA treatment, that caused an increase of soil temperature of about 3–4 °C (Fig. 2b), increased the dehydration rate of the thalli, and impeded a positive C balance in this treatment when light conditions were more favorable.

Although the data acquisition of our work was not extensive enough to quantify the relative importance of rain and NRWI in the biocrusts gas exchange, their variability,

frequency and timing characteristics suggest that reducing NRWI pulses suitable for carbon fixation could have a greater impact on the biocrust carbon balance at annual scale. For example, other studies performed in the desert of Neveg consider that dew is a more important source of water for biological activity than rain because (1) its lower inter-annual variability, 12 %, compared to 53 % for rainfall (Zangvil 1996); (2) its more frequency, 200 days of dew versus 20 days of rain (Temina and Kidron 2011); (3) and its well distribution over the year, a condition necessary for the formation of biological crust, as this implies that some moisture must be available on a regular basis (Jacobs et al. 1999). The important role of NRWIs in these dryland organisms has also been supported by several studies (e.g. Lange et al. 1994; del Prado and Sancho 2007; Kidron and Temina 2013).

During late autumn and winter, the F_v/F_m values observed in low biocrust cover plots were similar to those observed in high cover plots (Fig. 6d), indicating that the "bare" soil without a well developed and visible biocrust community possessed an abundant colony of free-living cyanobacteria and algae. This was consistent with our gross photosynthetic data (Fig. 6b). Cyanobacteria and algae constitute the early-successional stage during the development of biocrust communities (Garcia-Pichel et al. 2001, Lázaro et al. 2008), and have been described in worldwide dryland regions (Garcia-Pichel et al. 2003; Maestre et al. 2011). The positive effect of warming on the gross photosynthesis of low biocrust cover plots at Sorbas could have been produced because, when hydrated enough, free and lichenized cyanobacteria have a much stronger photosynthesis increase response to temperature than lichen species with green algae as photobiont (Lange et al. 1998; Housman et al. 2006). Dark respiration in low biocrust cover plots at Sorbas was significantly higher with warming in June (Fig. 6c), while this treatment did not affect dark respiration at high cover plots on any season. The respiration response to WA in free cyanobacteria, and the absence of it in green-algal lichens may indicate that the respiration of the first also had a greater sensitivity to temperature increase, or that the latter exhibited higher acclimatization capacity, as their fungal component possesses this ability (Lange and Green 2005).

Cyanobacteria possess a more efficient CO_2 concentrating mechanism (CCM) than green algae (Green et al. 1993; Badger et al. 2006; Price et al. 2008), which counteracts the increase of Rubisco oxygenase activity with temperature, leading to a photorespiration rise (Palmqvist et al. 1994; Smith and Griffiths 1998). Therefore, the CCM mechanism may be more beneficial at high temperatures, where this energy cost is smaller than the energy associated with photorespiration. This difference may also explain why cyanobacterial crusts are often the only biocrust type in arid and hyper-arid ecosystems, and their gross photosynthesis has a resilience to change at temperatures between 20 and 35 °C (Grote et al. 2010). It is also possible that under conditions of less NRWI formation, cyanobacteria have a competitive advantage to avoid desiccation over green algae lichens in terms of water-holding capacity (Lange et al. 1998), production of a protective layer of exopolysaccharides (De Philippis and Vincenzini 1998; Potts 2001), or even migration abilities through soil profile (Garcia-Pichel and Pringault 2001). These characteristics of cyanobacteria could explain why the drying effect of WA did not cause a negative response on the net photosynthesis of low biocrust cover plots (Fig. 6a).

Concluding remarks

Our results illustrate how the timing and magnitude of water pulses drive key ecological processes in dryland ecosystems, in this case, the carbon cycle linked to the metabolic activity of biocrusts. The shifts in these pulses caused by climate change will determine the magnitude of the effects on this biological activity, which will determine responses to

warming of the whole C cycle (Maestre et al. 2013). Our findings also emphasize the importance of NRWIs when understanding biocrust responses to climate change. They indicate that changes in NRWI regimes as consequence of warming could have a greater impact on the C balance of biocrusts than changes in rainfall amounts. Another contribution of this study is the differential response of the biocrusts to the climate change treatments as a function of their development stage. The results suggest that early-successional cyanobacterial biocrust could displace more developed lichen-dominated biocrusts in Mediterranean drylands, as indeed is happening in Aranjuez, the experimental site with more years of climate manipulation (Escolar et al. 2012). These changes induced by climate change have implications for the C cycle in the studied ecosystems, as early-successional biocrust areas have less C fix capacity. Consequently, the importance of dryland ecosystems as a C sink will be reduced if these shifts in the biocrust community take place.

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Easy-to-make portable chamber for *in situ* CO_2 exchange measurements on biological soil crusts

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Abstract

Commercial chambers for *in vivo* gas exchange are usually designed to measure on vascular plants, but not on cryptogams and other organisms forming biological soil crusts (BSCs). We have therefore designed two versions of a chamber with different volumes for determining CO_2 exchange with a portable photosynthesis system, for three main purposes: (*1*) to measure *in situ* CO_2 exchange on soils covered by BSCs with minimal physical and microenvironmental disturbance; (*2*) to acquire CO_2-exchange measurements comparable with the most widely employed systems and methodologies; and (*3*) to monitor CO_2 exchange over time. Different configurations were tested in the two versions of the chamber and fluxes were compared to those measured by four reference commercial chambers: three attached to two respirometers, and a conifer chamber attached to a portable photosynthesis system. Most comparisons were done on biologically crusted soil samples. When using devices in a closed system, fluxes were higher and the relationships to the reference chambers were weaker. Nevertheless, high correlations between our chamber operating in open system and measurements of commercial respiration and photosynthetic chambers were found in all cases ($R^2 > 0.9$), indicating the suitability of the chamber designed for *in situ* measurements of CO_2 gas exchange on BSCs.

Additional key words: chamber; cyanobacteria; infrared gas analyzer; lichen; moss; net photosynthesis; soil respiration.

Introduction

In the context of increasing interest in more accurate local and global carbon balance measurements, many studies have analyzed the conditions under which different plant functional types, such as broadleaf and needle trees, shrubs, and herbaceous C_3 and C_4 plants, are carbon sources or sinks (*e.g.*, Cox *et al*. 2000, Sitch *et al*. 2003, Friedlingstein *et al*. 2006, Magnani *et al*. 2007). However, the relationship with carbon acquisition or loss by gas exchange of biological soil crusts (BSCs), defined as communities of cyanobacteria, algae, mosses, lichens, and fungi in differing proportions, living on top of or within the first few millimeters of the soil surface, and closely bound to soil particles (Belnap *et al*. 2003), is still little known (Bowling *et al*. 2011). BSCs are especially important in the global CO_2 gas exchange in dryland ecosystems where they predominate and vascular plant productivity is limited (Lange *et al*. 1992, Lange 2003). Indeed, there is evidence of their major contribution to soil respiration in semiarid ecosystems (Castillo-Monroy *et al*. 2011), as well as their photosynthetic capacity under

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Abbreviations: BSC(s) – biological soil crust(s); [CO_2] – CO_2 concentration; IRGA – infrared gas analyzer; RH – relative humidity.
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appropriate moisture conditions (*e.g.*, Brostoff *et al*. 2005, Li *et al*. 2012). Global net carbon uptake by cryptogamic ground cover has been estimated at about 2.5 Pg yr^{-1} (Elbert *et al*. 2012), which corresponds to around 4.5% of net primary production by terrestrial vegetation.

Infrared gas analyzers (IRGAs) have been used for decades to study CO_2 exchange in several types of organisms, such as vascular plants, cyanobacteria, mosses, and algae (*e.g.,* Bloom *et al*. 1980, Dring and Brown 1982, Schipperges and Rydin 1998, Brostoff *et al*. 2002, Millan-Almaraz *et al*. 2009). There are two main IRGA operating systems: open (*i.e.*, where chamber air is renewed with ambient air from outside and CO_2 flux is calculated as the difference in CO_2 concentration ($[CO_2]$) between air in the chamber and incoming ambient air), and closed (*i.e.*, where air is not renewed from outside the system and CO_2 flux is calculated as the change in chamber $[CO_2]$ during the sample enclosure time). Both systems have advantages and disadvantages, which can be maximized and minimized, respectively, depending on a chamber design and configuration. The closed system was initially considered more accurate. It requires short measurement time, because, contrary to the open system, it does not need concentration gradient stabilization (Hutchinson and Mosier 1981, Sebacher and Harriss 1982, Mosier 1989, Vourlitis *et al*. 1993). This becomes more advantageous as the volume of the chamber increases (Hutchinson and Mosier 1981, Livingston and Hutchinson 1995). However, recently improved open system technology provides increased accuracy (*LI-COR* 2003, 2012), whereas the main disadvantage of the closed system, the gradual alteration of the initial concentration of gases inside the chamber over time persists (Nakayama 1990, Hutchinson *et al*. 2000, Davidson *et al*. 2002).

Although some studies have used chambers in closed systems (*e.g.*, Kershaw 1977, Snelgar *et al*. 1980), CO_2 fluxes in epiphytic, saxicolous, and terricolous lichens have been usually measured with chambers coupled to open system IRGAs, in *ex situ* controlled environments (*e.g.*, Carstairs and Oechel 1978, Lange *et al*. 1986, 1992; Friedmann *et al*. 1993, Zaady *et al*. 2000). These *ex situ* studies have provided valuable information for interpreting the responses of lichens and BSCs in their natural habitats (Lange *et al*. 2001), but they have not shown how far the laboratory response measurements differ from *in situ* responses. For instance, they require samples to be removed from their underlying soil, and there is little knowledge of how BSC removal (and often thallus clipping) affects the functioning of the component organisms. Even in field work, when the BSC removed from the soil is measured immediately in a cuvette, problems may linger due to the disruption of substrate water supply (Schroeter *et al*. 1999). In addition, these studies usually have a physiological approach, focusing only on BSC gas-exchange performance. However, in an ecological approach considering ecosystem implications, it is also necessary to take into account the contribution of the underlying soil, and therefore measurement of the whole soil profile becomes an advantage in quantifying the net ecosystem CO_2 balance. Despite these questions, there are few studies examining *in situ* net CO_2 exchange on biologically crusted soils, probably because the need for a more holistic approach to the subject has not come up until recent years.

As BSCs are very sensitive to humidity and temperature pulses (Grote *et al*. 2010, Bowling *et al*. 2011), reducing the enclosure time is critical to alter these two environmental factors as little as possible. Commercial canopy chambers which can be coupled to the soil surface, such as *LICOR* and *PP-Systems*, cannot deal with this problem properly, as their volumes are not adapted to short measurement times. To overcome this drawback, we have designed a robust, easy-to-make chamber for short *in situ* measurements of net CO_2 gas exchange on undisturbed BSCs, and tested two versions with different diameters.

This matter is currently arousing considerable interest, as shown by the growing number of studies based on customized chambers to be coupled to the soil surface (Bremer and Ham 2005, Botting and Fredeen 2006, Langensiepen *et al*. 2012). However, their conclusions are unclear because their different methodologies are not fully comparable. Therefore, the aim of this study was to standardize the *in situ* gas-exchange measurements on biologically crusted soils by (*a*) describing and discussing chamber design, and (*b*) determining its specific measurement configurations by comparison with widely used commercial chambers. Specific steps for this purpose were: (*1*) to find the best combination of input airflow rate and fan speeds (internal system fan and additional fan inside the chamber if needed) as well as the best operation measurement system (closed or open); (*2*) then with the best chamber configuration, to verify the validity of the chamber design for measuring CO_2 fluxes by increasing the number of regression points in system comparisons and extending their ranges; and finally, (*3*) to examine any possible microenvironmental disturbances that could be caused by our chamber, especially keeping in mind that the BSC gas exchange is particularly sensitive to changes in temperature and humidity.

Materials and methods

Description of the chamber: An 8.5-cm-high transparent chamber was constructed from methacrylate tubes (supplied by *Rotufer S.L.*, Almería, Spain; and *Decorplax Metacrilatos S.L.*, Madrid, Spain). Most chambers used for this purpose are made of methacrylate because of its uniform 92% transmissivity at 400–800 nm wavelength light, and because it is lightweight, low-cost, shockproof, and easy to handle and to bond with solvent glue (Bloom

et al. 1980). However, its water adsorption is high, which is a problem if in addition to photosynthesis there is interest in evapotranspiration. Therefore, to avoid adsorption/desorption of water vapor, the inner chamber walls were covered with a *Teflon*® film, which is also highly transparent in the 300–900 nm range (Kesselmeier *et al*. 1996, Cocker *et al*. 2001). The top methacrylate surface was designed flat instead of the spherical shape used in other commercial chambers for easier manufacturing and handling in the field, and to reduce the measurement time due to the lower chamber volume. The top can also be made with propafilm (provided by *LI-COR Biosciences Inc.*, NE, USA), but we preferred a hard cover to make it more robust.

As an interface between the chamber and the IRGA (portable photosynthesis system *LI-6400*; *LI-COR Biosciences Inc.*, NE, USA), we used a *9864-157* mounting plate (*LI-COR Biosciences Inc.*, NE, USA) from a *Conifer Chamber 6400-05*. A flat frontpiece of the same size as the metal plate was placed on the outside of the acrylic cylinder, and a methacrylate reinforcement was added inside the cylinder. A 0.5-cm-thick rubber gasket was added to the plate to seal the connection. We included an E-type thermocouple (not encapsulated) to record the surface temperature required for calculation of certain variables (such as vapor pressure deficit, total conductance to CO_2 and H_2O, or intercellular CO_2), as this temperature cannot be well estimated from air temperature with energy balance parameters in BSCs. An exhaust tube was inserted in a female hose fitting to route the air from the chamber to the match valve. The exhaust tube was placed at a 90° angle from the metal plate, far away enough to avoid shading. The chamber was designed to work on top of PVC collars previously inserted in the soil (*see* Castillo-Monroy *et al*. 2011 and Escolar *et al*. 2012 for this procedure). To ensure that the chamber was sealed on the soil, a toroidal disk covered with an air-tight rubberized band was placed between the chambers and the PVC collars inserted in the soil.

The use of different chamber diameters provides different possibilities, mainly related to the plot size required for each study target, whether almost isolated BSC species (small chamber) or BSC communities (large diameter chamber) are to be sampled. We therefore made two versions of the chamber with different diameters (Fig. 1), 10 and 20 cm [668 and 2,385 cm^3, respectively]. The large chamber had an additional 5 × 5 cm^2 fan inside, also at a 90° angle from the metal plate (opposite the match exhaust tube), to minimize shading and optimize air circulation, and to ensure that air entering the chamber was well mixed. It was powered by a 12 V-7A battery connected to a voltage regulator so that the optimal fan speed could be selected. The holes for inserting the thermocouple and the fan cable in the chambers were sealed with *Terostat-81*®, which is neither gas source nor sink.

Comparison with commercial chambers using BSCs under laboratory conditions: As further precautions must be taken to operate with larger volumes (for example, measurement times and proper air mixing are more critical), we started the two comparative tests of this section on the larger chamber. The tests were also conducted in the small chamber to ensure that the selected configuration was appropriate for its volume, but with some abbreviations based on the previous results. As the core of these tests was the selection of the best chamber configuration for measuring on BSCs, they were performed under controlled laboratory conditions, using unaltered soil samples with three types of BSC: with a cover close to 100% of (*1*) *Diploschistes diacapsis* (Ach.) Lumbsch lichen, (*2*) *Cladonia convoluta* (Lam.) Anders lichen, and (*3*) mosses (*Tortula* sp., *Crossidium* sp., and *Didymodon* sp.). PVC cylinder diameters of 10 cm (four samples for each crust type) and 20 cm (three replicates) were used to extract 6-cm-thick samples previously moistened to minimize damage to the crust. A brief description of the sampling sites can be found in the supplementary material (Figs. 1S, 2S - *available online*).

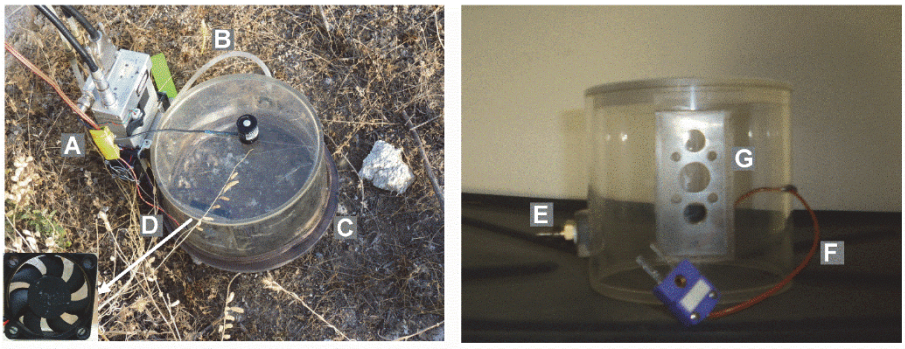

Fig. 1. *Left*: Large chamber with details of the fan voltage regulator (A), exhaust tube (B), toroidal disk (C), additional fan (D), with a detailed in the bottom left-hand corner insert. *Right*: Small chamber with details of the female hose fitting (E), thermocouple (F), and the flat frontpiece with three large holes matching the mounting plate, and another four for the screws (G).

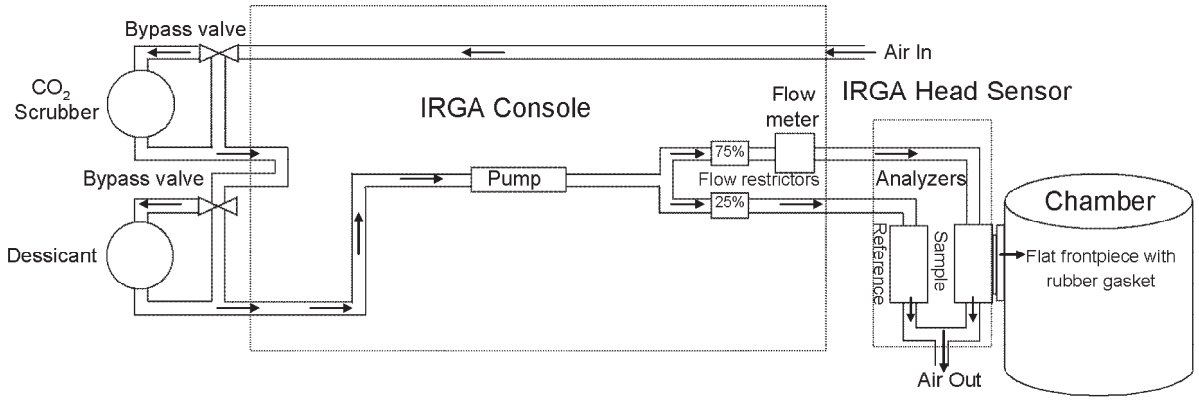

Fig. 2. Schematic flows in an IRGA connected to our chamber, based on *Li-6400/6400XT*. The IRGA system can modify CO_2 and H_2O concentrations in the incoming air by means of chemical tubes (circles). The pump speed controls the flow.

Prior to measurements, both small and large samples were moistened with 60 and 200 ml of distilled water, respectively, in order to enhance the biological activity of the soil, and thereby to gain a stronger respiratory signal. Measurements started at least 30 min after moistening to ensure steady CO_2 flux. *LI-6400* system flow and IRGA CO_2 zeros were calibrated before each test, and when this device was used in the open system (Fig. 2), sample and reference IRGAs were matched before each measurement. During these measurements, we covered the transparent chambers with an opaque cloth to prevent photosynthetic activity, and CO_2 flux recorded in each sample was related to the measurement immediately following it by an opaque commercial chamber attached to the soil respirometer *LI-8100* (*LI-COR Biosciences Inc.*, NE, USA) or *EGM-4* (*PP-Systems,* Hitchin, UK). The *LI-8100* was used for comparison to the two prototype chambers because it has two chambers with the same diameters as ours (10 and 20 cm). Only the small chamber was compared to the *EGM-4* respirometer, as this model only comes with a 10-cm-diameter chamber. Measurement time over the same sample was configured as 90 s, and data were stored every 5 s. The CO_2 flux measurements were adjusted to exponential and quadratic functions in the *LI-8100* and *EGM-4,* respectively, to acquire the final sample flux. The use of a linear function is not recommended, as gas diffusion theory predicts that the rate of gas exchange across the soil-atmosphere interface is not constant over the measurement period (Livingston *et al*. 2005). Absolute CO_2 flux was used for comparison between our chambers and commercial chambers, as the respiration and net photosynthesis systems have different signs.

Test 1. Determination of the best airflow and additional ventilation speed: With the larger chamber, always operating as an open system and with the internal fan at its highest speed, two airflow rates (300 and 700 μmol s^{-1}) were combined with three air speeds provided by the additional fan (0.7, 1.1, and 1.4 m s^{-1}). When the best combination of fan speed and airflow rate was found, based on the best fit of these measurements to the reference system (*LI-8100*), a last measurement was taken to check the effect of changing the speed of the sample IRGA's internal fan to low. Measurements were also performed without additional ventilation and with the best airflow rate previously found. In the small chamber, the airflow rate, additional and internal fan speed parameters were set according to the results of the large chamber comparisons, and the effect of additional ventilation was tested by comparing this chamber measurements, with and without fan, with those acquired by the *EGM-4* reference chamber.

Test 2. Open *vs.* closed system configuration: The respirometers used in this study operated in a closed system. In the IRGA attached to the prototype chambers (*LI-6400*), either open or closed system configurations may be selected. We tried both configurations with the samples, comparing their respiration responses measured by our custom chambers with those recorded by the *LI-8100* respirometer. The open system was configured with the best input airflow rate and fan speed combination found in the first test. All measurements in this system were taken after the gas exchange inside the chamber had stabilized (*i.e.*, the decimals in the photosynthetic rate were stable for 1 min). However, when measuring in the closed system, we used a procedure similar to that for the respirometers: the gas-exchange rate was recorded every 5 s for 90 s, and the final rate was found by regressing the individual rates over time. The fan speeds (additional and internal) were the same as for the open system, but in the closed system air was not recirculated by the pump.

Outdoor comparison tests: We carried out another two tests with the best chamber configurations found in the section above. The aim of these tests was to extend the data range of the regressions by measuring net photosynthesis under natural light conditions using the commercial *6400-05 Conifer Chamber* (*LI-COR Biosciences Inc.*, NE, USA) as the reference system, and increasing the number of points in the regressions using both respiration devices

for respiration measurements. The *LI-6400* calibration protocol and configuration of the respirometers were the same as in the section above.

Test 3. Net photosynthesis with our large chamber using vascular plants: As the *6400-05 Conifer Chamber* is unsuitable for soil measurements, we compared the CO_2 fluxes measured by our large chamber (without the opaque cloth) with this commercial chamber, operated by another *LI-6400* IRGA, using two vascular plants (*Ceratonia siliqua* L. and *Aspidistra* sp.). To close the custom chamber, a metal plate was placed on the bottom, and sealed with a gasket along the lower edge of the chamber wall. To eliminate the effect of any differences between the two *LI-6400s* in the comparison, both devices were previously compared using two of our large chamber prototypes with the same samples as in the preliminary comparison as well as with a vascular plant (*Aspidistra* sp.) to extend the measurement range. The small chamber was not used in this test.

Test 4. Respiration with our chambers using ruderal and biologically crusted soils*:* Our large chamber was compared to the *LI-8100* chamber under natural conditions. PVC collars (16) with a diameter of 20 cm were installed in the soil in an area with ruderal vegetation (weeds growing in human-disturbed fields). This soil was selected to widen the respiration range, as the previous crusted soils collected from semiarid ecosystems are characterized by low CO_2 efflux (Raich and Schlesinger 1992, Rey *et al*. 2011). Herbaceous plants within the collars were removed, and soil respiration disturbance caused by collar installation was allowed to stabilize for 24 h. Half of the collar enclosures were left with their natural moisture, and the other half was moistened with increasing amounts of water from 100 to 1,000 ml to acquire a wide range of respiration. Measurement started after 12 h, alternating plots with and without moistening, first with the prototype chamber connected to the *LI-6400*, which was covered to ensure darkness like the opaque respirometer chamber, and then with the *LI-8100* chamber. The measurements were carried out from 09:00 to 19:00 h (local time) in a total of six measurement cycles.

We compared our small chamber to an *EGM-4* chamber under seminatural conditions, *i.e.*, unaltered samples were *ex situ*, but measured outdoors. Samples of soil (12) with four kinds of BSC were collected, with three replicates for each type characterized by having a cover close to 100% of: (*1*) *D. Diacapsis* lichen, (*2*) *Squamarina lentigera* (Weber) Poelt lichen, (*3*) well-developed cyanobacterial BSC, including pioneer lichens, and (*4*) incipient-cyanobacterial BSC. An analysis of the cyanobacterial community composition was not performed in this study, but based on a recent publication (Büdel *et al*. 2014), it is likely that *Microcoleus* sp. and *Nostoc* sp. were present in these samples. The measurements were taken for two days, the first with the samples watered to saturation, and the second with the remaining moisture after 24 h of outdoor evaporation. Respiration of each sample (one cycle per day) was recorded with the prototype chamber connected to the *LI-6400* (also covered with an opaque cloth), and immediately afterwards with the *EGM-4* chamber.

Testing the influence of the chamber on micrometeorological variables: As a change in natural air pressure can directly affect gas diffusion in the soil-atmosphere interface (Kanemasu *et al*. 1974, Schlesinger 1977), the relationship between airflow rate and/or additional ventilation and the pressure inside the large chamber must be carefully supervised. And, as mentioned above, BSCs are very sensitive to humidity and temperature micrometeorological variables, thus any effect of the sample enclosure on these variables should also be checked.

Test 5. Check for changes in air pressure inside the chamber: The pressure inside the large chamber was monitored under laboratory conditions by inserting an *MSR 145* pressure sensor (*MSR Electronics GmbH*, Henggart, Switzerland) in the chamber. The threshold, where pressure changes affect BSC CO_2 measurements, was also tested by generating an artificial overpressure inside the chamber. To cause this overpressure, an additional airflow introduced through the thermocouple orifice was provided by a bottle with 509.5 ppm [CO_2]. The [CO_2] of the flow generated by the *LI-6400* was regulated by the *6400-01* CO_2 injector, and was the same as the extra airflow to avoid changes in the reference [CO_2] due to the use of two different flow sources. The enclosure effect in the small chamber was tested by selecting the best configuration found in the previous tests and without artificial overpressure.

Test 6. Check for changes in air temperature and relative humidity (RH) inside the chambers: This test was performed in the large chamber under field conditions from dawn to noon on a summer day to cover wide natural temperature and solar radiation ranges. A *DS1923 iButton® Temperature/Humidity Logger* (*Dallas Semiconductor*, Texas, USA) was placed next to the large chamber and another one inside it, both directly over the BSC surface. Since soil water content on that test day was low (on average, 4% at a depth of 5 cm), and therefore, evaporation was also low, we used a series of measurements taken with the prototype chamber on several sunny days with the high soil water content (on average, 21% at a depth of 5 cm) as recorded on data loggers (model *U23-001, HOBO Pro V2 Temp/RH Data Logger*, *Onset Computer Corporation*, Bourne, MA, USA) placed 20 cm above the soil surface. In this case, the chamber air RH measured by the *LI-6400* was compared with data logger records. The temperature test was not done in the small chamber, since the most influential factors in its change

(material spectral properties and air renewal) were the same as in the larger one. RH changes in the small chamber were checked using the *LI-6400* RH sample sensor records from Test 4 and environmental RH data from a nearby weather station provided by the Spanish Meteorological State Agency *(*AEMET*)*.

Results

Test 1. Determination of the best airflow and additional ventilation speed: The use of a low airflow caused that the flux measured in our large chamber was lower than the *LI-8100* respirometer data, which was three-fold higher (Fig. 3). In all cases, the data acquired in our large chamber were lower than those with the *LI-8100* respirometer, but this difference could be reduced by raising the airflow to a ratio of 1.5:1. Increasing the speed of the additional fan worsened the correlation between the two CO_2 flux measurement systems. The configuration with a slow fan speed had the best R^2 using both high and low airflow rates. We selected a high airflow rate with slow fan speed as the optimum combination because the measurements were nearest the reference system, the R^2 was high, and the chamber stabilization time was half that required at the low airflow rate. Therefore, the effect of the sample IRGA mixing fan speed was tested only with these optimal parameters. Best results were found with the IRGA fan at high speed (Table 1S – *supplementary material available online*; Fig. 4*A*), since although its regression was below the 1:1 line, their slopes were closer. We also found that, when the internal fan speed was lowered, R^2 fell from 0.83 to 0.59. The regression models fitted well with either low additional fan speed or without a fan, but with the first, *LI-6400* data were slightly higher and closer to those of *LI-8100* (Fig. 4*A*). An additional fan placed inside the small chamber and set at slow speed generally did not raise the measurements compared to the mode without ventilation (Fig. 4*B*), indicating that there were no problems of homogenization of the sample air in the absence of an additional fan.

Test 2. Open *vs*. closed system chamber configuration: In the large chamber, with the airflow and fan speed optimized, the coefficient of determination between the *LI-6400* and the *LI-8100* (respirometer) was substantially higher in the open system than in the closed system. In the open system, the regression slope was similar to the

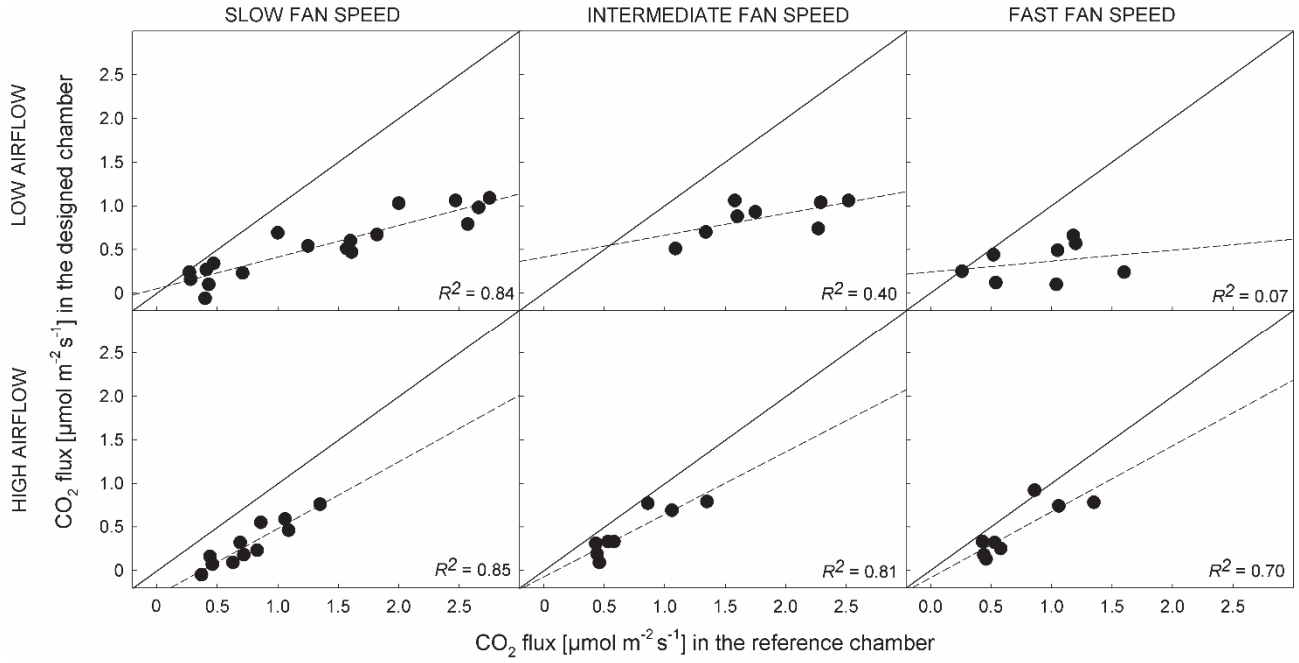

Fig. 3. Relationship between the respiration measured by our large chamber, attached to an *LI-6400*, and the reference system (respirometer *LI-8100*), at different airflow rates and additional fan speeds. *Dashed lines* – linear regressions, *solid lines* – ideal 1:1 relationships. Airflow rates were: low = 300 µmol s^{-1} and high = 700 µmol s^{-1}. The fan speeds were: slow = 0.7 m s^{-1}, intermediate = 1.1 m s^{-1}, and fast = 1.4 m s^{-1}. The measurements were performed under laboratory conditions (≈ 20ºC, moistened BSC samples), and with the sample IRGA internal fan set at high speed.

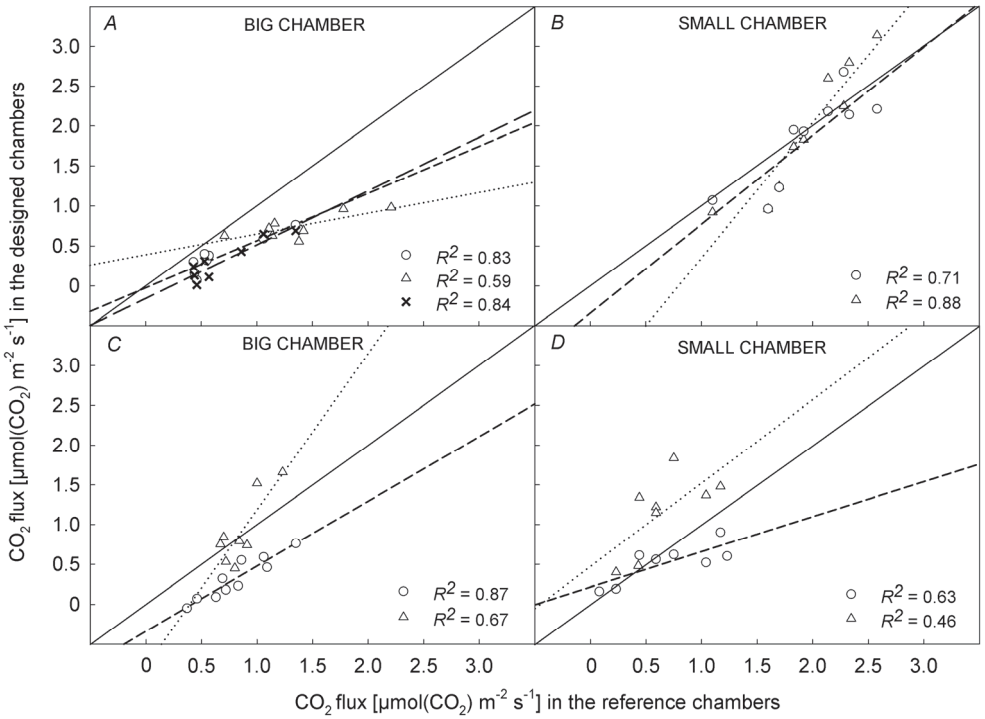

Fig. 4. Comparison of our in-house designed chambers attached to an IRGA *LI-6400*, and commercial chambers (reference chambers), under laboratory conditions (≈ 20ºC, moistened BSC samples). Solid lines – ideal 1:1 relationships. *A*: our chamber operating in the open system with the IRGA sample cell internal fan on high (*circles* and *short dashed line*) and low (*triangles* and *dotted line*) speed and the additional chamber fan on low (0.7 m s^{-1}). Measurements without the additional fan (*crosses* and *long dashed line*) and with the internal fan on high speed. *B*: the *LI-6400* in the open system with fan at low speed (*circles* and *dashed line*), without additional fan (*triangles* and *dotted line*), and internal fan on high speed. *C*: The *LI-6400* in open (*circles* and *dashed line*) and closed (*triangles* and *dotted line*) systems, both with fan on slow speed. *D*: the *LI-6400* in open (*circles* and *dashed line*) and closed systems (*triangles* and *dotted line*), both without fan. All open-mode measurements were done at an airflow rate of 700 µmol s^{-1}. The *LI-8100* respirometer was the reference system in *A*, *C*, and *D*, and the *EGM-4* device in *B*.

1:1 line, although the CO_2 flow was underestimated (Fig. 4*C*). In the small chamber, the R^2 coefficient was also higher in the open than that in the closed system (Fig. 4*D*). Nevertheless, CO_2 records in both systems were lower and higher, respectively, than respirometer data.

Test 3. Net photosynthesis measured with the large chamber *vs.* a conifer photosynthesis chamber: The large custom chamber measuring photosynthesis in the open system fitted well (R^2 = 0.959) to the commercial conifer chamber, with very similar measurements on the same samples, albeit slightly higher (Fig. 5*A*).

Test 4. Respiration under natural and seminatural conditions: The larger number of replicates and wider data range improved the fit between the two systems, resulting in a better correlation. The CO_2 flux in the *LI-6400* continued to be lower than in the *LI-8100*, although less than in the preliminary tests (Fig. 5*B*).

As in the test with the large chamber, the increase in replicates and respiration range by using samples with different moisture levels over time improved the fit between our small custom chamber measurements and the *EGM-4* respirometer (Fig. 5*C*). Measurements in both chambers were very similar even though the devices were from different manufacturers and operated in different configurations (open and closed systems).

Test 5. Changes in air pressure inside the chambers: The pressure variation in the chambers did not exceed 150 Pa when the IRGA system operated with its own airflow system, and with the fan at low speed (large chamber) or without the additional fan (small chamber). Pressures ranged (including the initial pressure without closing the chambers) from 101.47×10^3 to 101.58×10^3 Pa in the larger chamber, and from 100.82×10^3 to 100.85×10^3 Pa in the small chamber. Thus a high airflow in this system did not cause any pressure deficit or overpressure. When air from outside the system forced a pressure increase, the CO_2 flow remained unaltered as long as it did not exceed 200 Pa, but when it reached 300 Pa, the net CO_2 flow rate increased (Fig. 6*A*). The variation in pressure at different additional fan speeds were far from achieving the threshold needed to alter natural CO_2 flow rates (Fig. 6*B*).

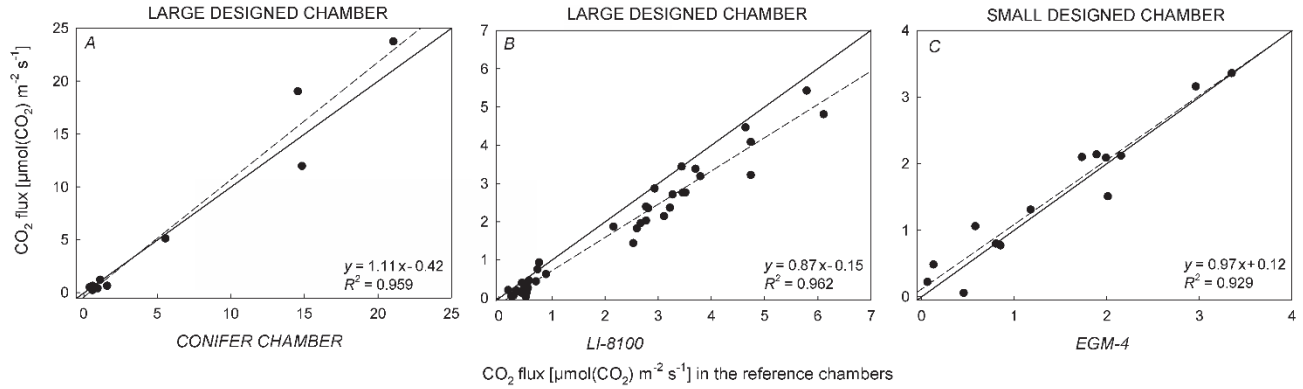

Fig. 5. Comparison of our in-house designed chambers attached to an IRGA *LI-6400*, and commercial chambers (reference systems) at outdoor conditions. *Dotted lines* – linear regressions; *solid lines* – ideal 1:1 relationships. *A*: comparison in the net photosynthesis range with vascular plants. *B*: comparison of respiration in the range extended by using ruderal soil samples. *C*: comparison of respiration using BSC samples. Selected *LI-6400* configurations: in *A* and *B*, IRGA sample cell internal fan at the highest speed, additional fan at low speed (0.7 m s^{-1}), and airflow rate at 800 µmol s^{-1}; in *C*, IRGA sample cell internal fan at the highest speed, without additional chamber fan, and airflow rate at 700 µmol s^{-1}.

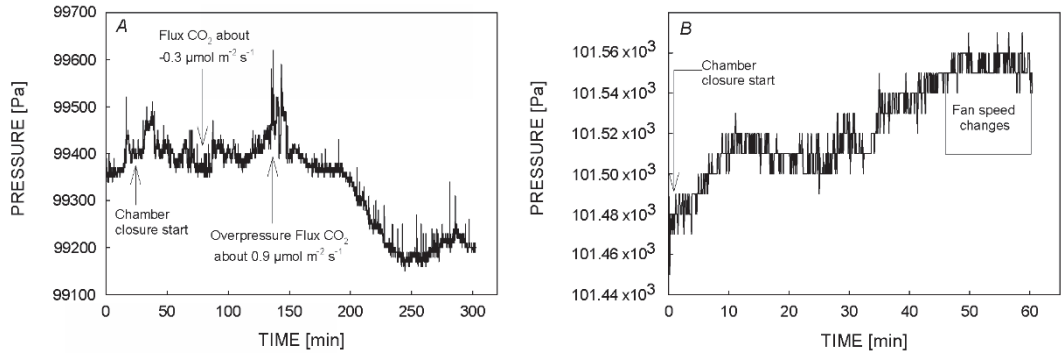

Fig. 6. Air pressure test. *A*: Effect of increased pressure on CO_2 flux. Airflow rate in the *LI-6400* was 800 µmol s^{-1}. *B*: Pressure in the large chamber during enclosure with changes in additional fan speed and 800 µmol s^{-1} airflow rate. Note that gas exchange is net assimilation: positive values imply a reduction in CO_2 concentration inside the chamber headspace, and negative values an increase.

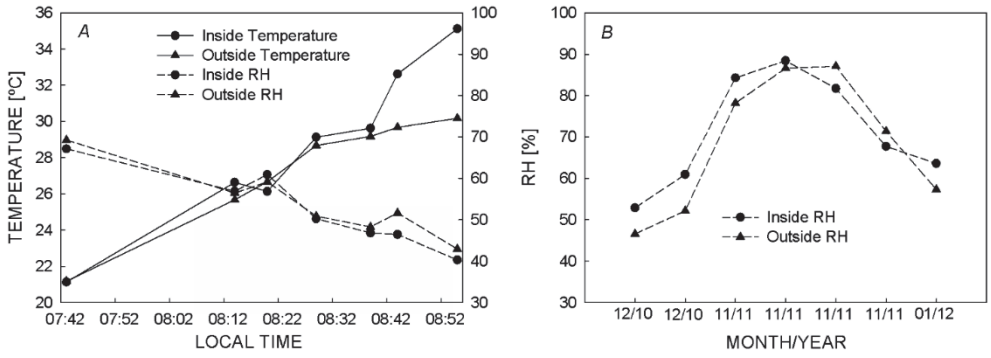

Fig. 7. Temperature and RH test. *A*: Temperature and RH inside and outside the large chamber with stabilized CO_2 concentrations (on average in 4 min) measured on a sunny summer day. *B*: RH inside and outside of the large chamber on sunny days and with high soil water content.

Test 6. Changes in temperature and RH inside the chambers: The paired measurements (outside-inside the chamber) showed that the air temperature inside the large chamber did not change with exposure to direct sunlight at temperatures below 30ºC, even though chamber closure time was over 4 min (Fig. 7*A*). However, when outside temperatures reached 30ºC, with the same closure time, the air temperature inside the chamber rose by as much as 5ºC. No relevant changes in air RH were observed inside the large chamber, where the average increase was only 3% in

summer, late autumn, and winter (Fig. 7*A*,*B*). However, in the small chamber, a considerable increase in RH was observed at high evaporation rates and when the soil moisture content was high, close to saturation; RH inside the chamber ranged from 37 to 94%, while the environmental RH only varied from 33 to 68%. See the supplementary material for a table summarizing all test results (Table 2S – *available online*).

Discussion

We have designed a versatile, robust, portable, and transparent custom-built chamber for *in situ* net CO_2 exchange on soils covered by BSCs in two different sizes, and compared it with other systems. Internal air movement and system mode (open or closed) were configured to standardize their measurements with other commercial chambers. The configurations with the highest determination coefficients were considered the best, their gas exchange rates could be equated with those of commercial chambers by a correlation equation, and with minimal instrumental error.

The CO_2 exchange measured in our chambers for soil respiration or net photosynthesis in vascular plants is quite similar to the measurements with various widely used commercial chambers. The laboratory and seminatural conditions to which the biologically crusted soil samples were exposed can be compared in terms of temperature and moisture (the most important environmental variables for the soil respiratory activity) with the growing season in their areas of origin. The ranges of the reference systems in our tests carried out on BSCs [0.1–3.3 μmol(CO_2) m^{-2} s^{-1}] were similar to those reported for *in situ* measurements during the growing season of one of these areas. For example, Rey *et al*. (2011) measured soil respiration on the same type of soil as our samples, in a range of 0.5 to 2.4 μmol(CO_2) m^{-2} s^{-1}. On a soil moisture gradient from near 0 to 25%, and including different types of soil in this area, the respiration flux varied from 0.6 to 3.8 μmol(CO_2) m^{-2} s^{-1} (Oyonarte *et al*. 2012). These ranges are lower than, for example, those found in subhumid forest ecosystems (*e.g.*, Rey *et al*. 2002, Tang *et al*. 2009, Arevalo *et al*. 2010), but similar to those observed in semiarid regions (Chen *et al*. 2008, Almagro *et al*. 2009, Castillo-Monroy *et al*. 2011). Therefore, according to the reference systems, our chambers were configured within a range of naturally-occurring dryland fluxes.

We discuss the results of our comparative tests further below, in the context of the conclusions of several previous studies. Although the literature describing the design of chambers for measuring soil respiration is quite abundant, there is not as much on design for net photosynthesis. We also address some special considerations which must be taken into account for these measurements.

Additional ventilation and airflow configuration: The optimal CO_2 flux measurement was found with high air intake (700–800 μmol s^{-1}) and the additional fan at low speed (0.7 m s^{-1}), but with IRGA sample cell ventilation at high speed (Figs. 3, 5). Our additional fan at high speed caused the worsened correlation between the two systems, possibly due to the generation of turbulence causing increased measurement randomness. Other authors have also recommended the use of a low fan speed (*e.g.,* Norman *et al*. 1997), as the turbulent airflows generated by high speeds decrease the soil boundary layer (Le Dantec *et al*. 1999, Janssens *et al*. 2000, Pumpanen *et al*. 2004). However, it is important to ensure adequate air mixing in the chamber. In the large chamber, the data recorded without ventilation were slightly lower than when moderate ventilation was used, showing that the additional fan in the chamber improved air mixing. In the small chamber, additional ventilation for proper air mixing was unnecessary, since data were no lower without fan action (Fig. 4*B*). In fact, the measurements with added ventilation had a lower R^2 and a stronger bias with respect to the *EGM-4*, possibly because the fan may have altered the aerodynamic conditions inside the chamber. This agrees with the *LI-COR Biosciences Inc.* recommendation that additional ventilation should be used when the volume of the chamber is over 2 L (*LI-COR* 2003).

In the open system, the steady-state [CO_2] gradient inside the chamber always differ from the outside natural conditions, but this disturbance can be minimized by optimizing the airflow generated by the system (Livingston and Hutchinson 1995). Although high airflow rates may lower these gradient differences (Gao and Yates 1998), they may also reduce measurement accuracy (*LI-COR* 2003). However, according to our results, the highest *LI-6400* airflow rate did not cause this problem with the chamber volumes employed, as demonstrated by their high correlation with the respirometers (Fig. 5). This was probably because the volumes were large enough in both cases to prevent the excessive air renewal that could cause CO_2 sample dilution to exceed sensor sensitivity levels (Ryden *et al*. 1978). Lower CO_2 fluxes, when low airflow was used in our large chamber, also reflected an air mixing problem. In addition, the high airflow rate shortened stabilization times (on average 2 and 4 min for the small and large chambers, respectively). This is especially important in poikilohydric organisms, for which enclosure times must be limited to a minimum due to the sensitivity of their metabolism to microenvironmental changes (Lange *et al*. 1997).

Comparison of systems under indoor conditions: We found a better correlation between the closed system *LI-8100* and our chamber in the open than in the closed system (Fig. 4*C*,*D*). CO_2 flux found with the *LI-6400* in

the closed system was higher than that in the open system. This was not consistent with the results found by Pumpanen *et al*. (2004) or Norman *et al*. (1997), who reported lower values in closed systems than in open systems. We do not think the lower CO_2 flux found in the open system was a result of poor air mixing, as we also found slightly lower values than with the *LI-8100* at a high additional fan speed. In addition, we consider the basis for closed systems inadequate for measuring the response of photosynthesis in a wide range of environmental conditions, precisely because of impossibility to maintain those environmental conditions constant as a consequence of the absence of air renewal (Field *et al*. 1989, Gao and Yates 1998). For example, in soil respiration, if the sample residence time is too long for a particular C efflux, the natural gas exchange in the soil-atmosphere interface decreases because of accumulation of the target gas in the chamber space (Rolston *et al*. 1976). This is one of the main problems of closed systems, and has been discussed in several articles (Ryden *et al*. 1978, Hutchinson and Mosier 1981, Mosier 1989, Rolston *et al*. 1976, Nakayama 1990, Freije and Bout 1991, Hutchinson *et al*. 2000, Davidson *et al*. 2002).

In fact, the open system has been used mainly for measuring photosynthesis in plants, whereas the closed system, cheaper and easier to handle, is used more frequently for soil gas fluxes (Healy *et al*. 1996, Conen and Smith 2000). This is probably because stronger changes in CO_2 and H_2O concentrations inside the chamber in the closed system affect the physiology of plants (*e.g*., stomatal conductance and photosynthetic rates) more than soil respiration measurements. Thus, we conclude that the open system is the best operating mode for measuring net photosynthesis with our chambers.

Comparison of systems under outdoor conditions: The similarity of the *LI-6400* (operating in the closed system) and the *LI-8100* soil respiration measurements has already been confirmed (Madsen *et al*. 2008). However, this study showed that this similarity continues to be valid when the *LI-6400* is operated with our custom chambers in the open system, considered a more appropriate method for measuring photosynthesis. Our two chambers were found to be consistent with the commercial chambers for both respiration and positive net photosynthesis over a wide range. The small chamber data were neither under nor over *EGM-4* respirometer measurements. However, the large chamber data were slightly lower than the *LI-8100* respirometer. Nevertheless, a high correlation between our chambers and the commercial respirometers and photosynthetic chamber measurements was found in all cases (Fig. 5, $R^2 > 0.9$), thus indicating the suitability of the custom chambers designed for measurement of CO_2 gas exchange on biologically crusted soils. Examples of its usefulness for measuring *in situ* net and gross photosynthesis can be found in Maestre *et al*. (2013) and Ladrón de Guevara *et al*. (2014).

Changes in micrometeorological conditions inside the chamber: One of the main open-system biases in the measurement of CO_2 flux could be caused by the generation of pressure differences between the chamber space and the ambient due to air circulation inside the system (Rayment and Jarvis 1997, Gao and Yates 1998, Welles *et al*. 2001). Soil porosity enables CO_2 to escape from the soil, where it is much more concentrated than in the air (from 2,000 to 10,000 ppm in soil, compared to 350–380 in the air, according to Welles *et al*. 2001). But, although CO_2 flux inside the chamber is less sensitive to overpressure than to pressure deficit, overpressure could lead to substantial underestimation of natural CO_2 flux (Fang and Moncrieff 1998). This can be seen in Fig. 6*A*, where overpressure caused a positive net assimilation flux, which indicates a reduction of [CO_2] inside the chamber headspace. It should also be considered that both overestimation and underestimation of CO_2 flux are higher in dry soils (Hutchinson and Mosier 1981, Lund *et al*. 1999), because water in soil prevents gas transport through the profile by advection, and greatly reduces molecular diffusion (Livingston and Hutchinson 1995). It is therefore particularly important to consider whether the chamber operating configuration increases or decreases air pressure when used on dry soils.

No relevant pressure change was observed when measuring in the open system (Fig. 6*B*), which shows that pressure deficit problems can be avoided by placing the pump before the air inlet into the chamber (such as in the *LI-6400*, *see* Fig. 2). But the most important factor that prevents important pressure changes is that the *LI-6400* pump is underpowered for this. Although the optimal airflow found in this comparison was at the pump upper limit (1.34 L min^{-1}), according to Fang and Moncrieff (1996), this airflow hardly alters the atmospheric pressure inside the chamber, regardless of whether by suction or pumping, or the length and diameter of air inlet and outlet tubes. Moreover, we found that the use of additional small fans with a slow speed in the large chamber did not cause any change in pressure.

Temperature rises inside the chambers because of the greenhouse effect of the material used, which can potentially directly and indirectly affect photosynthetic activity. Such increases change the affinity for O_2 of the primary carboxylating enzyme, Rubisco, and increases evaporation from the soil surface, decreasing the magnitude and duration of BSC hydration (Grote *et al*. 2010). However, in our 4-min-long field measurements, on sunny days, the temperature inside our custom chamber only increased when the outside temperature was over 25ºC. When the maximum outside temperature reached 30ºC, the temperature in the chamber was about 5ºC higher (Fig. 7*A*). The smaller temperature increase in this study may be due to ventilation and air renewal at a high airflow (800 µmol s^{-1}), as the use of a static-closed chamber could cause an increase in air temperature of about 15ºC in only three minutes (Matthias *et al*. 1980).

Lichens with green algae as the photobiont have been found to be able to recover metabolic activity at high relative air humidity without any other source of water (Lange *et al*. 1986, 1992). Thus, when soil evaporation is greater than the air renewal within the chamber, an increase in RH takes place in it, potentially causing artificial metabolic activation of some species in the BSC. Chamber measurements may thus overestimate the photosynthesis of poikilohydric organisms, as found by Wilske *et al*. (2008) in field measurements on BSC. It is therefore important to control RH when small chamber volumes are used. In our large chamber, no relevant changes in RH were observed due to the stronger air recirculation, and to the chamber volume, which acted as a buffer (Fig. 7*A*,*B*). However, under high evapotranspiration conditions, the small chamber was close to air water vapor saturation. The humidity input in the small chamber therefore needs to be regulated under extremely high moisture content and solar radiation conditions, which can be achieved by turning the *LI-6400* desiccant adjustment screw to scrub position, keeping H_2O vapor from entering the circuit (Fig. 2).

Conclusions and usefulness of the chamber: Our chamber, measuring in the open system mode, at high airflows and with the sample IRGA mixing fan at the highest speed, was demonstrated to be very suitable for measuring CO_2 exchange on soils with BSCs in a wide range of both positive and negative values. Custom manufactured chambers, such as those proposed here, have five main advantages: (*1*) they are cheaper than commercial chambers; (*2*) chamber diameters can be adapted to the best plot size for the study target (isolated species or communities); (*3*) the chamber volume can be minimized for faster measurement and fewer changes in the environment near the sample surface; (*4*) due to the shortened measurement time, more complete experiments with larger sample sizes can be designed; (*5*) and furthermore, our chamber can also be used as a respirometer simply by covering it with an opaque cloth. On the other hand, different chamber sizes also require different precautions for their proper application. For example, our larger chamber has to have additional low-speed ventilation for adequate air sample homogenization; and as our smaller chamber is more sensitive to changes in humidity, it requires manual adjustment of the humidity input for measuring under a high soil water content and evapotranspiration demand. Our results also demonstrated that this methodology is consistent and appropriate for use under field conditions, and the good correlations found with commercial respirometers mean that, for example, these two systems may be used simultaneously to estimate gross photosynthesis.

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Abstract: Changes in precipitation patterns affecting both rain quantity and its temporal distribution have been predicted for the Mediterranean Basin. This three-year study was performed to determine the physiological response of Macrochloa tenacissima, a dominant species in the Western Mediterranean grasslands, to these changes. A rainfall manipulation experiment was therefore conducted to test the interaction of two factors: quantity (Q) and frequency (F) of rainfall, both at three levels (100%, 75% and 50% of natural rainfall). A mobile transparent polycarbonate rain-out shelter was designed to cover experimental plots of M. tenacissima when it rained, and then treatments were implemented by watering. Reductions in Q and F caused seasonal down-regulation of net photosynthesis (A) and stomatal conductance (gs), but the first variable showed greater resistance to change. At annual scale, only F led A to lower rates. The decrease in Q and F had opposite effects on intrinsic water use efficiency (IWUE), enhancing and diminishing it, respectively. However, the response to Q was stronger, even exceeding the range of natural inter-annual variability. No rainfall reduction treatment decreased Fv/Fm regarding ambient treatment. In conclusion, although the responses to the simulated rainfall patterns did not surpass the current seasonal oscillations of M. tenacissima's physiological parameters, it did cause a down-regulation of its gas exchange and increase its water use efficiency.

Anexo: copia original

Highlights

- *M. tenacissima* used a water control strategy as response to rainfall shifts.
- Rain frequency played a more important role than rain amount in gas exchange shifts.
- IWUE increased with the reduction in rain amount, but decreased with its frequency.
- No rain shift treatment adversely affected F_v/F_m when compared to ambient treatment.
- The main driver of gas exchange for this species was the marked climate seasonality.

Climate change effects in a semiarid grassland: physiological responses to shifts in rain patterns

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Abbreviations: Q, rainfall quantity; F, rainfall frequency; *A*, net photosynthesis at leaf-scale; g_s, stomatal conductance at leaf-scale; IWUE, intrinsic water use efficiency; F_v/F_m, maximum photochemical efficiency of photosystem II; PAR, photosynthetically active radiation; ITRV, inter-treatment response variability; ISRV, interseasonal response variability; IARV, inter-annual response variability.

ABSTRACT

Changes in precipitation patterns affecting both rain quantity and its temporal distribution have been predicted for the Mediterranean Basin. This three-year study was performed to determine the physiological response of *Macrochloa tenacissima*, a dominant species in the Western Mediterranean grasslands, to these changes. A rainfall manipulation experiment was therefore conducted to test the interaction of two factors: quantity (Q) and frequency (F) of rainfall, both at three levels (100%, 75% and 50% of natural rainfall). A mobile transparent polycarbonate rain-out shelter was designed to cover experimental plots of *M. tenacissima* when it rained, and then treatments were implemented by watering. Reductions in Q and F caused seasonal down-regulation of net photosynthesis (*A*) and stomatal conductance (g_s), but the first variable showed greater resistance to change. At annual scale, only F led *A* to lower rates. The decrease in Q and F had opposite effects on intrinsic water use efficiency (IWUE), enhancing and diminishing it, respectively. However, the response to Q was stronger, even exceeding the range of natural inter-annual variability. No rainfall reduction treatment decreased F_v/F_m regarding ambient treatment. In conclusion, although the responses to the simulated rainfall patterns did not surpass the current seasonal oscillations of *M. tenacissima*'s physiological parameters, it did cause a down-regulation of its gas exchange and increase its water use efficiency.

Keywords: *Macrochloa tenacissima*; *Stipa tenacissima*; gas exchange; intrinsic water use efficiency; F_v/F_m; Mediterranean.

1. Introduction

Although the predicted changes in rainfall are subject to higher uncertainty than temperature (De Castro et al. 2005), climate change models for the Mediterranean region of Europe have found robust signs indicating a decrease in both quantity and number of annual rainfall events. It is also expected changes in their seasonal distribution and size, which will lead to a greater concentration of events in winter, more prolonged droughts in summer and an increase in extreme events (Giorgi and Lionello 2008). The effects of annual rainfall amount on vegetation have been studied extensively (e.g., Le Houérou and Hoste 1977; Aronson and Shmida 1992; Epstein et al. 1997; Knapp and Smith 2001). However, evidence of the importance of other rainfall pattern components on ecosystem responses to climate, such as seasonal timing, frequency and intensity of precipitation and drought length have increased since the start of the century (e.g. Bates et al. 2006; Chou et al. 2008; Knapp et al. 2008).

Rain manipulation experiments in hyperarid, arid, semiarid and dry-subhumid ecosystems (drylands) have been rarely implemented until recent years (e.g., Yahdjian and Sala 2006; Thomey et al. 2011; Tielbörger et al. 2014). However, this fact is not an indication of lack of interest, as they occupy ~ 41% of the terrestrial surface, contain ~ 46% of global carbon (C) reserves (Safriel and Adeel 2005), support ~ 50% of livestock (Puigdefábregas and Pugnaire 1999), and water availability mediates the responsiveness of these ecosystems to global change (Huxman et al. 2004). Plant community responses to changes in rainfall patterns vary according to the characteristics (such as phenology, morphology and physiology) of the component species (Robertson et al. 2010). *Macrochloa tenacissima* (L.) Kunth (=*Stipa tenacissima* L., alpha grass, esparto) is a rhizomatous, C_3 perennial tussock grass widespread and endemic in Western

Mediterranean drylands (Gutiérrez 2000), and one of the few species which is usually dominant in its community. So exploring how this species responds to changes in rainfall can reflect the direction of the responses at community level. In addition, this species is ecologically important, not only for its spatial predominance, but also due to its morpho-physiological adaptation to this semiarid climate (Ramírez 2006). Xerophytic communities in Mediterranean grasslands must endure different gradients of water, light and temperature stresses caused by harsh seasonal variations (Madon and Médail 1997). The *M. tenacissima* strategy for coping with wide variability in abiotic stress, especially related to water availability, is seasonal changes in its physiological parameters (e.g. Domingo et al. 1991, 2003; Haase et al. 1999; Balaguer et al. 2002; Ramírez et al. 2008). In light of the above, the purposes of this three-year study were to: i) evaluate *M. tenacissima* plant-scale ecophysiological responses to changes in rainfall patterns (amount and frequency of precipitation); ii) explore the consistency of these responses on two time scales (seasonal and annual), and any change in their importance; iii) determine whether the responses to the simulated rainfall patterns could exceed variability in the response to the current rainfall pattern. To pursue these objectives, we designed a full-factorial rainfall exclusion experiment in which two factors were manipulated, rainfall quantity (Q) and frequency (F). Ecophysiological responses of *M. tenacissima* to the treatments were monitored by measuring its leaf-scale gas exchange (CO_2 and H_2O) and maximum PSII photochemical efficiency (F_v/F_m) after each rainfall event for a continuous three-year period.

Previous studies on *M. tenacissima* suggest that a decrease in rainfall will limit its C assimilation (Haase et al. 1999), but it can tolerate prolonged drought because it is able to withstand extreme leaf dehydration (Balaguer et al. 2002). In addition, this species can respond quickly to individual water pulses to partly recover gas exchange

activity after severe water stress (Pugnaire et al. 1996). These physiological features, as well as apparent coupling of leaf water content and soil moisture, have led to its classification as a functional poikilohydric species (Balaguer et al. 2002). Hence, we hypothesized that: i) the predicted changes in rainfall patterns cause down-regulation in net photosynthesis (*A*), stomatal conductance (g_s) and F_v/F_m; ii) severe water stress increases the instantaneous intrinsic water-use efficiency (IWUE), as in a previous study in which this variable increased sharply with decreasing g_s (Ramírez et al. 2009); iii) response to change in rainfall could surpass the current inter-annual response range, but this is less likely for interseasonal response since *M. tenacissima* is a drought resistant species with several water control mechanisms, one of them consisting in reducing gas exchange near zero in the season with increased water stress (Haase et al. 1999; Ramírez et al. 2009).

2. Materials and methods

2.1. Study site

Rainfall manipulation was done at the Balsa Blanca experimental site, an *M. tenacissima*-dominated grassland with gentle topography located in the Cabo de Gata Natural Park, Almería, SE Spain (N36°56′26.0″, W2°01′58.8″) at 200 m a.s.l. The climate is semiarid warm Mediterranean, with prolonged summer droughts. Mean annual precipitation and temperature are ~ 220 mm and 18ºC, respectively, with strong intra- and inter-annual variability in precipitation. This rainfall is far from enough to compensate the potential evapotranspiration (~ 1390 mm). The soil at Balsa Blanca is classified as Calcaric Mollic Lithic Leptosols (IUSS Working Group WRB 2006). It is

shallow, of variable depth (down to a maximum of ca. 0.3 m, but on average 0.1 m), stony, alkaline (pH > 8) and with carbonate saturation that has led to the formation of a petrocalcic horizon. This horizon is highly permeable due to its porosity and the presence of fissures and fractures (Rey et al. 2012).

The vegetation is dominated by *M. tenacissima*, which represents ~ 80–85% of the cover of vascular plants (Oyonarte, pers. comm.). This ecosystem also includes xeric shrubs (e.g., *Phlomis purpurea* L., *Thymus hyemalis* Lange, *Thymelaea hirsuta* L., *Ulex parviflorus* Pourr.), some other grasses (*Brachypodium retusum* Beauv.) and scattered individuals of climactic shrubs (*Chamaerops humilis* L., *Olea europaea* L. var. *sylvestris* Brot., *Rhamnus lycioides* L.). Annual plants show considerable biodiversity, although cover is usually low. There are no trees, and lichen (*Diploschistes diacapsis* Lumbsch., *Cladonia convoluta* Cout.) or moss-dominated biocrusts often occupy plant interspaces. Vascular plant cover is ~ 55%, and according to Mora and Lázaro (2013), this vegetation has not undergone significant alteration since at least 1955.

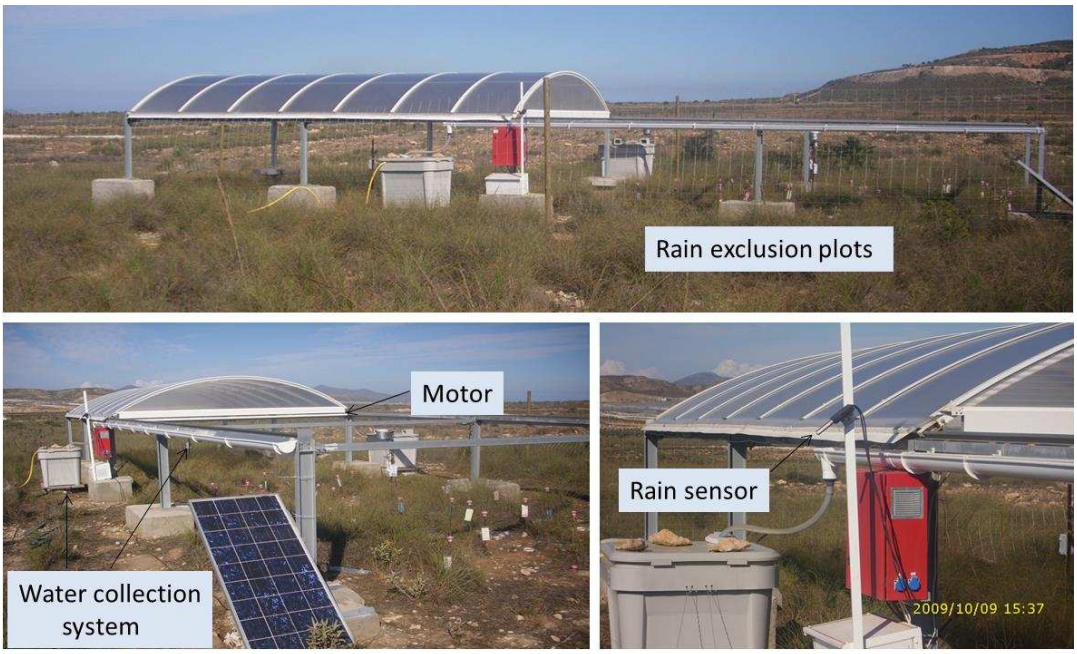
2.2. Experimental design, setup and effects on microclimate

A full-factorial rainfall manipulation experiment was setup at Balsa Blanca, to test the Q and F factors in February of 2009, excluding all natural rainfall with an automatic mobile shelter, and performing manual irrigation treatments after each rain event. The reduction in precipitation in the Mediterranean Basin forecasted by climate models has a wide seasonal range of ~ 15%-20% annually, but up to 30%-45% in the driest season (Bates et al. 2008; Giorgi and Lionello 2008; Mariotti et al. 2008). We set three levels in each factor (100%, 75% and 50% of the natural rainfall during the experimental period) since use of more than two factor levels along a resource gradient

149 is recommended to test different fitness or ecophysiological response scenarios (Feng
150 and van Kleunen 2014). Crossing the two factors and three levels made nine treatments,
151 each with three replicates, so 27 *M. tenacissima* tussocks were used in plots shaped and
152 sized to the plants. The replicates of each treatment were applied to a small (< 0.12 m^2),
153 medium (0.12–0.25 m^2) and large (> 0.25 m^2) tussock to include all the size and age
154 variability in the site's natural population (Figure S1). Uniform distribution of these
155 parameters during the treatments was considered important since previous studies have
156 revealed that small and juvenile tussocks are more vulnerable to water stress and their
157 physiological performance is different from mature tussocks (Armas and Pugnaire
158 2005; Ramírez et al. 2008). The plots were carefully selected to fulfill the following
159 criteria: (i) minimal area including 27 variable-sized tussocks; (ii) easy access for
160 construction of the shelter; (iii) very little slope to avoid distortion of irrigation
161 treatments by runoff.

162 The mobile 40 m^2 transparent polycarbonate shelter with a rain sensor was
163 specially designed to automatically cover the plots when it rained, and uncover them the
164 rest of the time to minimize micrometeorological disturbances (Figure 1). The shelter
165 was open-sided with a curved roof having a minimum and maximum height of 1.55 and
166 2.40 m, respectively, to ensure enough air circulation underneath it and thus prevent a
167 greenhouse effect when it was over the plots. Two methacrylate plates were also added
168 on the frontal sides to minimize entry of rain to the rain-out zone. Shelter orientation
169 was north-south to minimize shading on the plots by the structure. Two rain gauges with
170 HOBO dataloggers (Onset Computer Corporation, Bourne, MA, USA) were installed,
171 one outside the shelter and another inside, the latter recording precipitation entering in
172 the rainfall exclusion zone before it was covered by the shelter. All the precipitation that
173 was kept from entering the rainfall exclusion zone was collected by two gutters along

174 the sides of the shelter and stored in two 500 l tanks. This water was used for the rain
175 treatments, which were applied with a sprinkler (to simulate natural rainfall), between
176 the first and third day after each rainfall event.
177



178

179 **Figure 1.** Mobile rain-out shelter showing its most important components.
180

181 Two criteria were established for defining an event: i) rainfall events had to be at
182 least two days apart, the time required to perform treatments and ecophysiological
183 measurements, and ii) there had to be at least 2 mm of rainfall, because below this
184 threshold canopy interception loss in this species is nearly 100% (Domingo et al. 1998).
185 The amount of water to be used for treatments was calculated as the difference between
186 the two rain gauges, and the mm of rain were converted to ml for watering taking into
187 account the area of each plot. The F factor was run with days without watering
188 treatment and days with compensatory watering to maintain the full amount of Q (see
189 explanation in Table 1).

Table 1

Example of F factor irrigation schedule according to natural rainfall events.

Events	F100	F75	F50
1			
2			Events 1 and 2
3			
4			Events 3 and 4
5		Events 4 and 5	
6			Events 5 and 6
7			
8			Events 7 and 8
9		Events 8 and 9	

White cells: days with irrigation treatment; lined cells: days without irrigation treatment; grey cells: days with compensatory irrigation, i.e., sum of the amount of two consecutive rain events with their corresponding Q factor percentages.

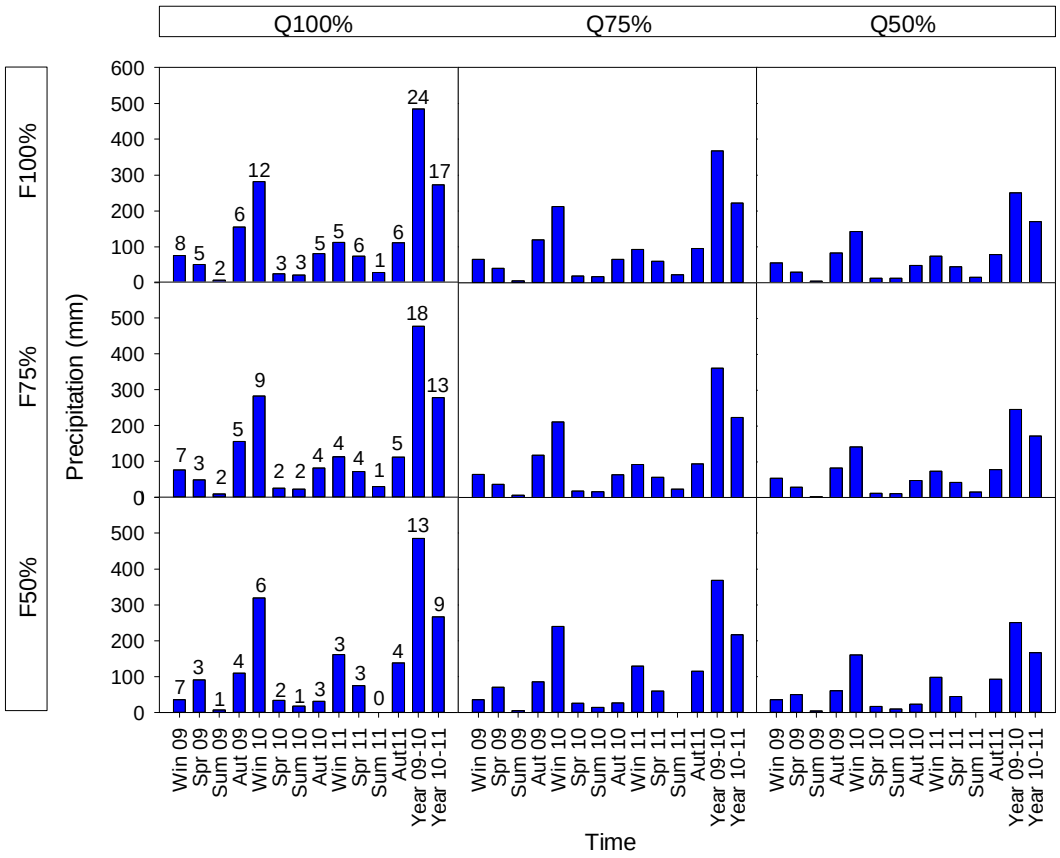
To avoid water losses or gains unrelated to the treatments, in addition to installing the plots in a place with very little slope (ca. 2–4º), they were delimited by 20-cm-high metal plates driven into the ground as deeply as possible. As a third precaution, the treatments with the least possibility of generating runoff were placed at the top of the slight slope, whereas the treatments that could generate more runoff because of the concentration of rain in fewer events (e.g., Q100-F50) were at the bottom (see Figure S1). In addition to the shelter treatment plots simulating natural ambient (Q100-F100; 'ambient treatment'), we selected another ten plants (reference plots) outside the rainfall exclusion zone (for logistic reasons, 300 m away from the shelter) encompassing the full range of sizes and ages. In these plants, the same physiological monitoring as those under the shelter was performed to determine whether the response of the plants with controlled watering was similar to that of plants under natural rainfall.

To monitor micrometeorological conditions and assess the effects of the shelter on the plots, we measured air and soil temperatures, air relative humidity (RH) and photosynthetically active radiation (PAR) in the rainfall exclusion zone and in the area covered by the shelter when it was not raining. Surface soil temperature (0–0.02 m) and PAR were continuously monitored using RT-1 and QSO-S sensors, respectively (Decagon Devices Inc., Pullman, WA, USA), and air temperature and RH using VP-3 sensors (Decagon Devices Inc.). These data were collected on two Em50 Digital/Analog Dataloggers (Decagon Devices Inc.) and averaged every 5 min. These micrometeorological measurements showed that PAR was the variable most altered by the shelter, causing ~ a 50% reduction on sunny days (Figure S2A) and ~ 20–30% on cloudy days (Figure S2B). However, as only covered the plots during rainfall events, the average seasonal reduction was 14%, 6.4%, 1.6% and 6.6% in winter, spring, summer and autumn, respectively. The increase in soil temperature under the shelter was only relevant in the early hours of the afternoon in summer, rising around 8ºC (Figure S3A), because this is the season with the highest natural temperatures (Figure S3B). However, this is the season that the shelter covered less time the experimental plots due to the rainfall regime. As the open sides allowed natural air circulation, the air temperature only rose from 1 to 2ºC under the shelter in all seasons. RH tended to be slightly lower under the shelter, but the difference was only ~ 3%.

2.3. Natural rainfall pattern during the experiment and performance of the treatments

Precipitation during the two hydrological years was above the long-term average of 220 mm. The first hydrological year was exceptionally wet, doubling this

241 average (Figure 2). This deviation from the mean occurred only once in the long-term
242 data (1961–1990; data source from AEMET, Spanish Agency of Meteorology). The
243 second hydrological year was close to the long-term average, but also surpassed it.
244 Winter was the season with the most rainfall, and summer with the lowest, which is
245 the normal pattern in Mediterranean semiarid ecosystems. Q treatments were close to
246 the annual experimental target (Figure 2), but with a downward deviation due to some
247 shelter displacement failures. In the F75 and F50 treatments, quantities were
248 concentrated on fewer rainy days, causing events of greater magnitude and intensity
249 (Figure S4). The distribution of the events also had a maximum in winter and a
250 minimum in summer, with the highest seasonal number of events in winter of 2010,
251 the rainiest period during this experiment, and the lowest in summer of 2011 (Figure
252 2).



253
254 **Figure 2.** Irrigated water (mm) per treatment and time interval (seasons and

hydrological years). The numbers above the bars in the left section denote the number of rainfall events per frequency level and time interval.

2.4. Ecophysiological measurements

Ecophysiological measurements of the reference plants were done on the same day that irrigation treatments took place. After watering, we waited the same time that had elapsed between the rainfall event and data collection on the reference plants to perform the measurements on the treated plants. This elapsed time regarding watering (from one to three days later) is when the response to water pulses is maximal in *M. tenacissima* (Pugnaire et al. 1996). In both cases (shelter internal and external plants), the measurements were taken at the time of highest photosynthetic activity of the day, which was estimated according to previous full-day cycle measurements in different seasons (unpublished data). Due to the high uncertainty of being able to correctly place humidity sensors in a shallow soil with high presence of stone fragments, and the possibility of causing damages in the roots, soil water content was not recorded in the plots. Therefore, this measurement schedule was considered the appropriate to relate ecophysiological responses with accurate irrigation data and to better discern the different responses to treatments in a functional poikilohydric species.

Leaf gas exchange measurements were performed using an infrared gas analyzer (LI-6400; LI-COR, Lincoln, NE, USA) coupled to a chamber with an area of 6 cm^2 and an LED light source (model 6400-02B, LI-COR). CO_2 and H_2O zeros and flow meters were calibrated before each data collection. Natural input air was taken at two meters above the soil surface by a tube and a 6-L bottle was used as a buffer volume before the air entered into the system. Prior to measurement in each day, PAR and block chamber

280 temperature were set to standardize the environmental conditions during data collection.
281 PAR corresponded to a sunny day and the chamber temperature was the natural air
282 temperature at start of measurement (dataset range: 800–2000 μmol m^{-2} s^{-1} in PAR; 17–
283 30ºC in temperature). System airflow was regulated according to the photosynthetic
284 activity, with a minimum of 200 μmol s^{-1} at very low activity (near compensation
285 point), and a maximum of 350 μmol s^{-1} at high activity, and was also fixed at the
286 beginning of the measurements of each day. During the measurements, sample and
287 reference infrared analyzers were matched about every 15 min. With these records,
288 three ecophysiological variables were acquired: *A*, g_s and IWUE. *A* and g_s rates were
289 estimated with a leaf area correction, calculated as a flat surface when the leaf was not
290 rolled up, and as a semi-cylinder when the leaf rolled up due to water stress. In each
291 plot, from four to six leaves were placed in the chamber, all facing north to reduce the
292 PAR variability incident on the samples prior to their introduction into the chamber.
293 Only mature leaves were used because they represent the largest area with
294 photosynthetic activity all year around (78–100% of plant green area) and they usually
295 have a slightly higher photosynthetic rate than young leaves (Haase et al. 1999). The
296 measurements were performed in the longitudinal middle area of the leaves to reduce
297 the within-leaf gas exchange variability (Ocheltree et al. 2013). IWUE was calculated as
298 a ratio of A/g_s.

299 After gas exchange measurements, F_v/F_m was recorded on the same leaves with
300 a Handy PEA fluorometer (Hansatech, Kings Lynn, UK). Dark-adapted F_v/F_m reflects
301 the potential quantum efficiency of PSII and has been used extensively as a sensitive
302 indicator of plant photosynthetic performance (Maxwell and Johnson 2000). Therefore,
303 prior to measurement, the leaves were adapted to the dark with leaf clips (Hansatech,
304 UK) for 30 min. Measurements using a saturating light pulse of 3000 μmol m^{-2} s^{-1} took

5 s. Before measuring, autogain was set to maximise the signal during measurement to ensure that the samples did not exceed the instrument scale (Hansatech Instruments 2006).

2.5. Data analyses

Prior to the analysis, seasonal and annual averages were calculated for each variable and plot using the dataset of event measurements. Annual analyses were performed by hydrological years (from September to August of the following year), since this represents both the natural cycles of rainfall and plant species activity. The analyses of the four physiological variables (*A*, g_s, IWUE and F_v/F_m) were implemented in InfoStat (ver. 2013p, http://www.infostat.com.ar.), a user-friendly interface for R (ver. 2.15.2). General linear mixed models (LMMs) with repeated measures were used to test seasonal and annual differences in the physiological variables among the treatments.

Q, F, Time (season or year) and their interactions were included in the models as fixed factors, and the plots were entered as a random factor on the intercept to reduce the effect of intraspecific variability of the responses to the treatments. The effects of entering leaf temperature (LT) in the *A* models, and PAR in the *A*, g_s and IWUE models as covariates were also tested. These covariates were recorded by the LI-6400 system at the same time as gas exchange measurements, and were also entered in the model as seasonal or annual averages. Only when the covariates had a significant effect at $p < 0.05$ were included in the models, and if both of them were selected in the case of the *A* models, possible multicollinearity between them was analyzed using the variance inflation factor (VIF) parameter. Analysis of annual F_v/F_m

required rank transformation of the data for a normal distribution. In each model selected, Fisher's least significant difference (LSD) post-hoc test was applied to the most complex interactions of the factors showing significant differences for all pairwise comparisons. This procedure was also performed to analyze and compare the physiological activities of the ambient treatment and reference plots.

Inter-treatment response variability (ITRV) was analyzed in the four physiological variables and compared to the natural interseasonal and inter-annual response variability (ISRV and IARV, respectively) to the current climate ambient treatment. ITRV was estimated as the mean rate of change (calculated for each plot size) between the ambient treatment and the treatments with the strongest response (i.e., the treatments with the maximum or minimum mean in each period analyzed). ISRV and IARV were estimated as the mean ambient treatment rate of change (also calculated separately in the three plots) between seasons and years, respectively. *T* tests were performed to determine whether the ITRVs were significantly different from the ISRV and IARV. When the variances were not homogeneous, power transformations were used.

3. Results

3.1. ISRV and IARV of M. tenacissima under the ambient rainfall pattern

In gas exchange variables, ISRV was greater than ITRV, as ambient treatment had rates of change between the seasons with maximum and minimum *A* and g_s of 79.60% and 79.92%, respectively, in 2009-10, increasing to 95.01% and 85.85% in

2010-11 (Table A.1). ISRV rates in F_v/F_m were lower and more constant in the two hydrological years, 22.79% and 22.35% in the first and second year, respectively, whereas the most different ISRV rates between these two years were found in IWUE (20.04% in 2009-10, and 68.51% in 2010-11). The difference in rainfall between the two years is reflected in the average annual gas exchange, as in the first rainy year *A* and g_s were higher (Table A.2). However, despite these two very contrasting hydrological years, the IARV was four to five-fold lower than the ISRV. The IARV was negligible in IWUE and F_v/F_m.

3.2. M. tenacissima response to the rainfall reduction treatments

The main findings related to ecophysiological responses to changes in rainfall patterns are: i) g_s and IWUE were more sensitive to change than *A* at seasonal scale; ii) F factor played a more important role than Q factor in gas exchange shifts; and iii) no rainfall reduction treatment caused a down-regulation of F_v/F_m regarding ambient treatment. In the next sections we described the most complex interactions for each variable.

3.2.1. Net photosynthesis (A)

For *A*, on a seasonal basis, Q × F × Time interaction was found statistically significant (Table S1). The significant effect of the interaction took place in winter and autumn of the second hydrological year, in 2011 (Figure 3A). In both seasons, Q100-F100 had the highest *A*, but in winter only Q75-F75 was significantly lower than the ambient treatment, whereas in autumn there were four treatments with a significant decline in *A* (Q100-F75; Q75-F75; Q75-F50; Q50-F50). In this first

season, Q50-F100 and Q75-F50 also had significantly higher *A* rates than Q75-F75, so there was no linear relationship between factor reduction and *A* response. In the analysis of annual averages, only F and Time factors caused effects on this variable without interacting between them (Table S1). Reduction in F negatively affected the performance of *A* at 75 and 50 levels. Hydrological Year 2009-10 had higher average *A* than 2010-11, most likely as a result of higher rainfall in the first year (Figure 3B).

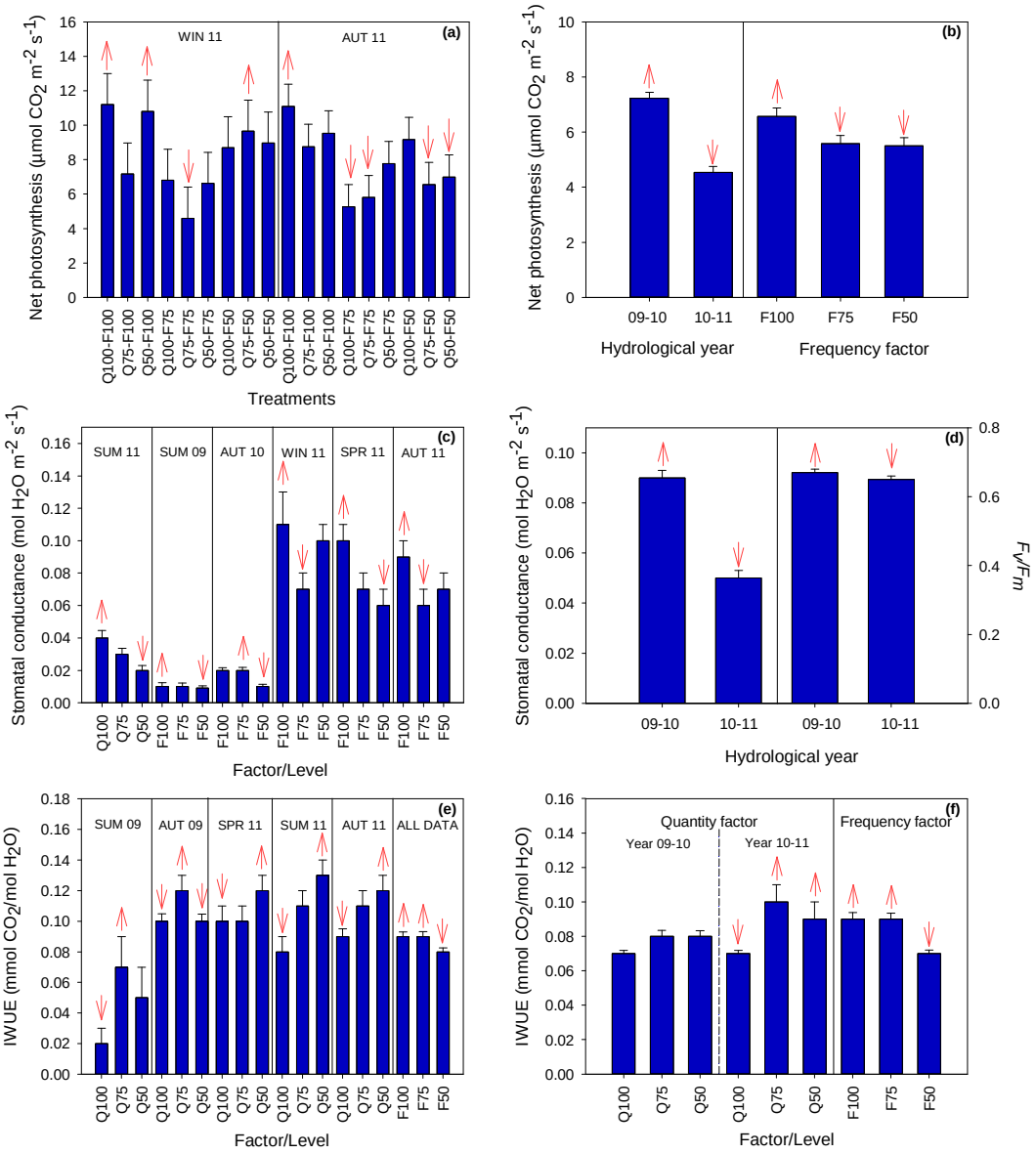


Figure 3. Fisher's least significant difference (LSD) post hoc results for seasonal and annual *A* (**A**, **B**), g_s (**C**, **D**) and IWUE (**E**, **F**). F_v/F_m means (**D**) show the results on an

annual scale. The most complex significant interactions of each factor in these variables and temporal scales are shown in the periods that took place. The main significant effects of the factors are shown when they did not have any significant interaction. Data are mean ± SE (n = 3). Upward arrows indicate significantly higher means than those with downward arrows.

3.2.2. Stomatal conductance (g_s)

For g_s, Q × Time and F × Time interactions were found statistically significant in the seasonal analysis (Table S1). The F factor had more seasonal impact on this variable than the Q factor (Figure 3C) or the interaction of the three factors on *A* variable. Taking into account both factors, there were six periods with significant responses to the treatments. The ambient treatment had higher g_s than the 75% and 50% reductions in two and three periods, respectively. But in autumn of 2010, the 75% reduction was the level in F with a greater g_s than the 50% reduction. No significant effect of Q or F was found in the analysis of hydrological years (Table S1). As in *A*, Hydrological Year 2009-2010 had higher average g_s than in the following year (Figure 3D).

3.2.3. Intrinsic water use efficiency (IWUE)

Seasonal Q × Time interaction and F as main effect were found statistically significant (Table S1). In the year 2009, IWUE at Q75 was significantly higher than at Q100, but in year 2011, the Q50 reduction level showed the best IWUE performance (Figure 3E). Winter was the only season without any significant effect, and after the rainy winter of 2010, no treatment had any effect in any season until spring of 2011. In the F factor, IWUE was significantly lower at F50 than the other levels (Figure 3E).

As on the seasonal scale, the Q factor led to significant annual differences through its interaction with Time, and the F factor was statistically significant without any interaction (Table S1). The Q factor only had an effect in the last year, when the IWUE increased in both Q reduction levels. In the F factor, the post-hoc result was the same as at seasonal scale, with a lower IWUE at F50 than the other levels (Figure 3F). Therefore, IWUE was sensitive to changes in intra-annual Q and F close to the long-term mean annual amount of precipitation, but only to F when the ambient amount of precipitation doubled the long-term mean.

3.2.4. Maximum photochemical efficiency of PSII (F_v/F_m)

As in variable *A*, a seasonal Q × F × Time interaction was found in F_v/F_m (Table S1). In the first measurement season (spring of 2009), the mean F_v/F_m with Q50-F75 irrigation was significantly higher than the means in all treatments with Q100 level and in Q50-F50 (Figure 4). Q50-F75 also had a significantly higher F_v/F_m than for Q75-F75 in autumn of 2011. In summer of 2010, the mean of F_v/F_m in Q50-F50 was significantly lower than in the rest of the treatments, except in those with Q100 water application. As in *A* and g_s variables, there was a significant difference between hydrological years in the Time factor, with no interaction with other factors (Table S1; Figure 3D). Neither Q nor F factors caused any response in the main effects or interactions at annual scale.

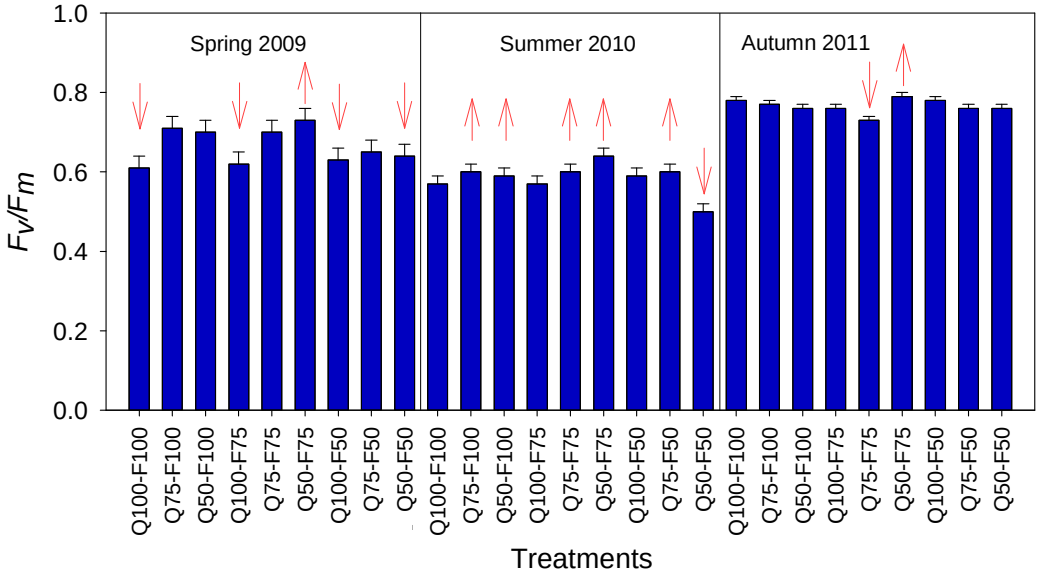


Figure 4 Fisher's least significant difference (LSD) post hoc seasonal-scale results for F_v/F_m. The Q × F × Time interaction is shown only with the period when it took place. Data are mean ± SE (n = 3). Upward arrows indicate significantly higher means than those with downward arrows.

3.3. Comparison of ambient treatment and reference plots

Seasonal physiological activity was similar in both ambient treatment and reference plots most of the time. However, in *A* and IWUE, there were three periods when statistical differences between these plots were detected, with an interaction between the Time and Plot-type factors (Table S2): Summer of 2009, winter of 2010 and autumn of 2011 in *A*, and summer of 2009 and autumns of 2010 and 2011 in IWUE (Figure S5A, C). In all these periods, except IWUE in autumn of 2011, the values of the reference plots were higher than for the ambient treatment plots. Differences in g_s were found only in winter of 2010 and summer of 2011. In the first case, g_s was higher in the reference plots, and in the second, in the ambient treatment

plots (Figure S5B). Although the results of the mixed model indicated a Plot-type × Time interaction in F_v/F_m (Table S2), significant seasonal differences these plots were not found in the post-hoc analysis (Figure S5D).

The analysis of means for hydrological years showed significant differences in *A* between years, but not between the types of plots (Table S2; Figure S6). In g_s the interaction Plot-type × Time was almost significant (Table S2), and a post-hoc analysis was performed because is considered advisable when the F value is over 2 and the sample size is small (Fry et al. 2013). This analysis showed that the reference plots in 2009-10 had significantly higher mean g_s than the rest of the means (Figure S6). In conclusion, the differences between ambient and reference plots detected in some periods could not be due to micrometeorology alterations by the shelter since it was over the plots only ~ 1% of the experimental time, but due to spatial variability of the physiological responses driven by different factors that determine water availability at plot scale.

3.4. Comparison of M. tenacissima ITRV with its ISRV and IARV

The *A* and g_s ITRV rates calculated by comparing the ambient treatment and treatments with the most down-regulation in each season were significantly lower than the ambient treatment rates of change between seasons in most periods (Table A.1). ITRV rates were calculated using the treatments with the highest values in IWUE and F_v/F_m variables, and in this case only two and four periods, respectively, had significantly greater ISRV (ITRV represented in column four, and ISRV in columns five and six of Table A.1). However, annual ITRV was significantly higher than the IARV in IWUE (columns four and five, respectively, of Table A.2). This

476 occurred in both hydrological years, although the strongest change in IWUE from a
477 rainfall reduction treatment was in the second year. In this variable, the rate of change
478 of the treatment causing the minimums was also calculated, as the F factor caused
479 down-regulation. In this case, the biannual means were used since F did not interact
480 with time. This rate of change was very close to the IARV rate, as was also the case
481 for F_v/F_m. ITRV in A and g_s doubled the IARV rate in the second hydrological year,
482 but this difference was not statistically significant.
483
484
485 **4. Discussion**
486
487 *4.1. Intra-seasonal and intra-annual effects of the simulated rainfall patterns*
488
489 Unexpectedly, Q50-F75 was the treatment with the highest F_v/F_m. This
490 treatment also had significantly highest values of NDVI parameter (data not show)
491 than the rest of the treatments. These two variables are sensitive to chlorophyll
492 content, especially in the low range of values and under drought conditions (Gitelson
493 and Merzlyak 1997; Guo et al. 2008). Hence, the most plausible explanation for this
494 result is that plants that of this treatment had at the beginning of the experiment higher
495 average chlorophyll content due to interindividual natural variability (see Figure 4,
496 where in the first season F_v/F_m of Q50-F75 is significantly higher than several
497 treatments). During the experimental period these differences were diminishing, but in
498 moments of increased drought, when chlorophyll contents are minimal, its influenced
499 over F_v/F_m was reflected again in lesser magnitude (F_v/F_m of Q50F75 was only
500 significantly higher than one treatment in two periods of drought) due to the higher

sensitivity of this parameter under this conditions. In conclusion, no rainfall reduction treatment adversely affected F_v/F_m parameter, pointing out the great resistance of *M. tenacissima* photosynthetic apparatus to water stress.

Performance of *A* in this species showed resistance to change in response to intraseasonal rainfall availability, as until winter of 2011 the treatments did not cause any effect (Figure 3A). However, this resistance was lower than in shrub species in this region according to Miranda et al. (2011), who only found significant gas exchange response after four years of 30% reduction in water and seasonal variation in precipitation, and significant differences between treatments disappeared in the extremely rainy Hydrological Year 2009-10. The fact that our experiment was also performed during this period probably facilitated this seasonal resistance to change in *A* rate. Most Mediterranean herbaceous species are able to maintain their physiological performance in an environment of irregular precipitation by using several water use efficiency control strategies (Moreno et al. 2008). Stomatal behaviour is the main mechanism plants have for controlling the trade-off between water loss and carbon gain, and their closure is among the first physiological events occurring in response to a decrease in water availability (Flexas et al. 2004). We thus found a g_s response to the treatments from the first experimental year, which was also the driver for rapid changes in IWUE (Figure 3C, E).

In line with studies that highlight the role of rainfall frequency behind shifts in ecosystem structure and functionality (e.g., Knapp et al. 2002; Büdel et al. 2009; Heisler-White et al. 2009), in our experiments, the F factor caused the greatest responses in the gas exchange variables (*A* and g_s). In *A*, a seasonal interpretation is more complex due to the three-term interaction, Q × F × Time. Supporting our initial hypothesis, one and four rainfall reduction treatments caused down-regulation of *A* in

winter and autumn of 2011, respectively (Figure 3A). However, in the winter of 2011, Q75-F50 had greater *A* rates than Q75-F75. Previous studies have found that more intense events, such as the one caused by our F reduction in irrigation, infiltrate more deeply into the soil (e.g., Weltzin et al. 2003; Loik et al. 2004; Knapp et al. 2008). This effect can benefit water availability in drylands, but in contrast, a decrease in F also causes an increase of days between events. Thus, depending on the number of events, their sizes, and their temporal distribution, there is a threshold where F no longer favours water availability but limits it instead.

In winter of 2011, the F50 distribution caused two big events (>20 mm) to be combined in one, whereas in F75, a big event combined with a small (≈5 mm) one, so rain intensification was negligible (see Figure S4) and the negative effect of increased days between events prevailed. This positive effect of event concentration probably only affected *A* in winter because the soil porosity is higher (Mora and Lázaro 2014) and the evaporative demand is lower (Serrano-Ortiz et al. 2014). But in autumn of 2011, Q mediated, causing a threshold in the negative effect of F50, with down-regulation at Q75-F50 and Q50-F50, but without any significant response at Q100-F50. On the other hand, F also mediated the response to Q, as in the F reduction treatments *A* suffered a decrease even maintaining the ambient Q. Q50-F75 was unaffected probably, as pointed out above, because of the effect of a greater chlorophyll concentration in the samples of this treatment.

As expected, g_s was down-regulated by reduction in both Q and F, but especially F (Figure 3C). In some periods (winter and autumn of 2011), the most stomatal closure was at Level F75, but in others (summer of 2009, autumn of 2010 and spring and summer of 2011) it was at F50. As in *A*, these responses suggest an effect of F on water availability which is complex and nonlinear. IWUE increased

551 with the reduction in Q (Figure 3E, F), because drought-induced stomatal closure
552 restricts water loss more than CO_2 uptake (Ramírez et al. 2009; Jongen et al. 2011).
553 However, Ramírez (2006) reported decreasing IWUE in *M. tenacissima* when soil
554 water content fell below 5%, and even it becomes negative if respiration surpasses
555 photosynthesis. This is consistent with our results, since in the driest periods (summer
556 09 and autumn 10) *A* was near the compensation point and ambient treatment IWUE
557 was at a minimum (Table A.1). The F50 level triggered a decline in IWUE, which is
558 in agreement with the results of Fay et al. (2011) in a mesic ecosystem, as they
559 suggest that most ecosystem process rate reductions due to increased variability in
560 rainfall is an indicator of lower ecosystem rainfall use efficiency. However, this
561 conclusion cannot be generalized, because in another Mediterranean-type ecosystem
562 with a higher rainfall regime than our study area, water-use efficiency is more related
563 to temperature (Jongen et al. 2014).

564 The timing and amount of the first rainfalls ending drought are important
565 attributes of Mediterranean precipitation patterns (Swarbreck et al. 2011), as they are
566 critical to triggering biological activity (Beatley 1974). This fact has been highlighted
567 in the present study, since it determined the effects of the treatment effects in autumn.
568 The results demonstrated that an extraordinarily large event of 85 mm at the
569 beginning of the autumn of 2009 buffered the deleterious effect of F reduction in *A*,
570 and only IWUE had a significant response to the treatments increasing the rate in Q75
571 level. In autumn of 2010, the situation was the contrary, as there was no rainfall event
572 over 10 mm until the end of the season. So the range of *A* was similar within all the
573 treatments during this period, because of the absence of any relevant rainfall to trigger
574 recovery. However, if the first event after summer is > 25 mm, as was the case in the

575 third autumn, all variables responded to the treatments, showing that the recovery
576 activity period is particularly sensitive to changing rainfall patterns.
577
578 *4.2. Will the physiological responses to future rainfall patterns exceed their current*
579 *range of interseasonal and inter-annual variability?*
580
581 The knowledge of the natural variation of a system is a necessary step in
582 understanding ecological processes and the implications of ecological changes
583 (Landres et al. 1999). One of the goals of this study was to determine whether the
584 shifts in rainfall patterns forecasted for the Mediterranean Basin are a force of change
585 powerful enough to drive its terrestrial ecosystems beyond their current range of
586 variability. Our results show that, although Q and F had significant seasonal effects on
587 all variables, the main driver of gas exchange activity for this species was the
588 characteristically strong seasonal shifts in precipitation and temperature of the
589 Mediterranean climate, rather than the simulated rainfall patterns.
590 The Mediterranean climate is characterized by strong seasonal distribution of
591 precipitation, with most of the rainfall concentrated in autumn and winter. Therefore,
592 the warmest months coincide with low rainfall, and *M. tenacissima* adapts its
593 metabolic activity drastically to the rising gradient of environmental stress (water
594 deficit, high temperature and irradiance) from mid-spring to early autumn with down-
595 regulation of gas exchange. Consequently, the course of time over the seasons caused
596 greater changes in the physiological variables (especially in gas exchange rates) than
597 the effects of treatments within each season (Table A.1). However, it must be
598 remembered that the experimental period did not cover the effects of the treatments in

599 an extremely dry year, and therefore we cannot extrapolate the findings of our study to
600 this precipitation range.

601 Unlike the seasonal scale, we found that ITRV was a greater force of change
602 than IARV in all variables except F_v/F_m (Table A.2). This demonstrates that intra-
603 annual variations in individual event size and the interval between the events
604 influence plant processes regulating carbon and water cycling more than the average
605 annual amount of precipitation (Schwinning and Sala 2004; Fay et al. 2008). In spite
606 of the wide average differences between ITRV and IARV, the statistical results were
607 not significant in gas exchange variables because there were fewer changes in g_s and
608 *A* in small and medium-size plots, respectively. Therefore, resistance to change in the
609 gas exchange variables due to the simulated rainfall patterns was plant-size specific.
610 In IWUE, the rate of change caused by the treatment with the highest value was out of
611 the range of the IARV, but this did not happen in the treatment with the lowest value.
612 This shows that, although IWUE had an opposite response to F and Q, this variable is
613 more sensitive to change in Q and its annual range will be shifted upward. However,
614 intra-annual response variability in this variable was significantly greater than inter-
615 annual in both years, indicating that IWUE was also sensitive to the distribution of the
616 water inputs along the year.

617 Another finding is that the magnitude of the change in response between
618 treatments differed in these hydrological years of contrasting amounts of rain.
619 Thomey et al. (2011) also found a differential inter-annual strength of ANPP response
620 to intra-annual Q and F rainfall changes in desert grasslands. Thus, our results support
621 the idea of Thomey et al. (2011) that it is not only important to predict relative
622 changes within years, but also patterns of responses between years.

623

5. Conclusions

The impact of potential changes in rainfall patterns will depend not only on the predicted scenarios of change, but on the way the ecosystem's dominant species have adapted under current climate conditions (Hanson and Weltzin 2000). For example, the physiological parameters of Mediterranean woody and annual species have shown great resistance to change under new rainfall pattern scenarios (Miranda et al. 2011; Jongen et al. 2013). In *M. tenacissima*, *A* only had significantly different responses to the treatments in WIN11 and AUT11, and g_s was primarily decreased in the last experimental year, consequently increasing IWUE. Thus, the existence of water use efficiency control mechanisms in this species was responsible of the resistance response to change *A* under different rainfall patterns.

The effects of the treatments observed in this experiment were mediated by the current seasonal rainfall distribution, as the recurrent interaction of the Q and F factors with Time implies (Table S1). These effects were complex in the gas exchange variables, probably because there were no linear relationships between the factor levels and their interaction with water availability. For example, under some circumstances F75 had a stronger negative seasonal impact on gas exchange than F50, but neither of them increased the rates over the current ambient rainfall pattern. In addition, in spite of the resistance to change detected at seasonal scale, both levels of frequency reduction caused an annual decrease in the *A* rate. Therefore, regardless of the magnitude of change in the factors tested, down-regulation of the gas exchange was detected, a point that must be taken into account in the quantification of carbon

and water balances in those areas where *M. tenacissima* is dominant, as well as its influence on novel dynamic climate models.

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APPENDIX

Table A.1
Comparison of the response variability caused by the reduction treatments and the natural interseasonal response variability of the ambient treatment.

Season	Ambient treatment	Treatment with maximum effect	% change treatment	% change with previous season	% change Max./min. season
		A(µmol CO_2 m^{-2} s^{-1})			
SPR 09	9.56	Q50-F100 / 6.56	21.67%	-	-
SUM 09	0.24	Q100-F50 / 0.15	56.67%	**97.67%**	-
AUT 09	6.48	Q75-F50 / 2.98	53.00%	93.04%	49.99% / 51.18%
WIN 10	9.56	Q75-F75 / 7.41	20.33%	30.89%	30.26% / **70.66%**
SPR 10	13.61	Q50-F100 / 12.63	22.33%	30.26%	0.00% / **79.60%**
SUM 10	2.74	Q50-F50 / 1.87	28.67%	**79.60%**	**79.60%** / 0.00%
AUT 10	0.64	Q100-F50 / 0.22	39.82%	78.86%	**95.01%** / 0.00%
*WIN 11	11.2	Q75-F75 / 4.60	49.70%	**95.01%**	0.00% / **95.01%**
SPR11	10.77	Q75-F50 / 5.51	40.84%	40.18%	40.18% / **93.63%**
SUM 11	4.58	Q50-F50 / 2.15	53.28%	46.23%	54.90% / **87.01%**
*AUT 11	11.09	Q100-F75 / 5.26	50.63%	58.09%	-
		g_s (mol H_2O m^{-2} s^{-1})			
SPR 09	0.10	Q50-F100 / 0.07	28.01%	-	-
*SUM 09	0.012	Q100-F50 / 0.011	65.39%	85.78%	-
AUT 09	0.07	Q75-F50 / 0.03	43.14%	76.23%	59.08% / 38.92%
WIN 10	0.12	Q75-F75 / 0.09	26.52%	36.31%	31.12% / **69.78%**
SPR 10	0.18	Q75-F100 / 0.15	14.78%	31.12%	0.00% / **79.92%**
SUM 10	0.04	Q100-F75 / 0.03	14.07%	**79.92%**	**79.92%** / 0.00%
*AUT 10	0.02	Q75-F100 / 0.01	33.33%	45.26%	**85.85%** / 0.00%
*WIN 11	0.14	Q75-F75 / 0.05	58.44%	85.85%	0.00% / 85.85%
*SPR11	0.13	Q75-F50 / 0.05	59.58%	34.15%	34.15% / **84.17%**
*SUM 11	0.05	Q50-F75 / 0.02	54.65%	59.65%	60.45% / 61.24%
*AUT 11	0.12	Q75-F75 / 0.06	48.89%	52.92%	-
		IWUE (mmol CO_2/mol H_2O)			
SPR 09	0.08	Q100-F50 / 0.10	11.46%	-	-
*SUM 09	0.03	Q75-F75 / 0.04	81.33%	71.69%	-
*AUT 09	0.09	Q75-F75 / 0.13	28.30%	**73.01%**	0.00% / 20.04%

WIN 10	0.080	Q75-F75 / 0.083	12.59%	13.26%	13.26% / 13.51%
SPR 10	0.08	Q75-F75 / 0.09	16.07%	8.63%	15.56% / 9.45%
SUM 10	0.07	Q50-F75 / 0.10	23.61%	9.45%	20.04% / 0.00%
AUT 10	0.03	Q75-F100 / 0.08	50.96%	60.49%	68.51% / 0.00%
WIN 11	0.08	Q50-F50 / 0.11	27.05%	**62.31%**	15.54% / **62.31%**
*SPR11	0.08	Q50-F75 / 0.13	39.50%	23.98%	21.11% / 58.81%
*SUM 11	0.09	Q75-F100 / 0.15	29.92%	21.11%	0.00% / 68.51%
*AUT 11	0.10	Q50-F75 / 0.12	15.57%	14.68%	-
			F_v/F_m		
*SPR 09	0.61	Q50-F75 / 0.73	15.81%	-	-
SUM 09	0.50	Q75-F75 / 0.54	18.59%	17.54%	-
AUT 09	0.73	Q75-F100 / 0.74	3.57%	**30.63%**	3.41% / **21.73%**
WIN 10	0.72	Q50-F50 / 0.74	3.91%	1.49%	2.47% / **20.96%**
SPR 10	0.74	Q50-F75 / 0.75	3.30%	2.47%	0.00% / **22.79%**
*SUM 10	0.57	Q50-F75 / 0.64	10.94%	22.79%	22.79% / 0.00%
AUT 10	0.55	Q100-F75 / 0.59	13.29%	10.08%	22.35% / 0.00%
WIN 11	0.71	Q100-F50 / 0.74	4.37%	22.35%	0.00% / 22.35%
SPR11	0.68	Q75-F100 / 0.71	13.93%	6.11%	6.11% / 18.87%
SUM 11	0.67	Q75-F100 / 0.69	3.81%	10.59%	6.46% / 20.75%
*AUT 11	0.78	Q50-F75 / 0.79	2.93%	**13.62%**	-

* denotes seasons with significant responses to the treatments in the LMM models. Treatments with maximum effect are those that caused minimum seasonal *A* and g_s, and maximum seasonal IWUE and F_v/F_m. The fourth column gives the rate of change from the ambient treatment to treatment with the maximum seasonal effect. The fifth column gives the rate of change between the ambient treatment and its value in the antecedent season. The sixth column gives the rate of change from the ambient treatment to its seasonal maximum and minimum within hydrological years. The seasonal rates of change of the ambient treatment are highlighted in black bold when are significantly higher (p values < 0.05) than the intraseasonal rates of change caused by the rainfall

826 reduction treatments (n = 3). Note that the % of change were not calculated with the
827 values of columns two and three, but as the averages of the % of change per plot-size.
828

Table A.2
Comparison of the response variability caused by the reduction treatments and the natural interannual response variability of the ambient treatment.

Years	Ambient treatment	Treatment with maximum effect	% change treatments	% change between years
		A(μmol CO_2 m^{-2} s^{-1})		
09-10	7.51	Q75-F75 / 6.50	12.83%	19.89%
10-11	6.21	Q75-F75 / 3.62	40.30%	19.89%
		g_s (mol H_2O m^{-2} s^{-1})		
09-10	0.10	Q75-F75 / 0.07	21.12%	20.51%
10-11	0.08	Q50-F75 / 0.04	45.15%	20.51%
		IWUE (mmol CO_2/mol H_2O)		
09-10	0.073	Q75-F75 / 0.09	**17.40%**	**4.02%**
*10-11	0.071	Q50-F75 / 0.11	**31.91%**	**4.02%**
*Both years	0.072	Q100-F50 / 0.070	3.93%	4.02%
		F_v/F_m		
09-10	0.672	Q50-F75 / 0.70	3.41%	2.50%
10-11	0.667	Q75-F100 /0.668	2.49%	2.50%

829

830 * denotes hydrological years with significant responses to the treatments in the LMM
831 models. Treatments with maximum effect are those that caused minimum annual *A* and
832 g_s, and maximum annual IWUE and F_v/F_m. "Both years" in IWUE refers to the
833 treatment with the minimum biannual average. The fourth column gives the rate of
834 change from the ambient treatment to the treatment with the maximum annual effect.
835 The fifth column gives the rate of change from the ambient treatment to its value in the
836 other year. The inter-annual rates of change of the ambient treatment are highlighted in
837 red bold when are significantly higher (p values < 0.05) than the intra-annual rates of
838 change caused by the rainfall reduction treatments (n = 3). Note that the % of change

were not calculated with the values of columns two and three, but as the averages of the % of change per plot-size.

Table 1

Example of F factor irrigation schedule.

Events	F100	F75	F50
1			
2			Events 1 and 2
3			
4			Events 3 and 4
5		Events 4 and 5	
6			Events 5 and 6
7			
8			Events 7 and 8
9		Events 8 and 9	

White cells: days with irrigation treatment; lined cells: days without irrigation treatment; grey cells: days with compensatory irrigation, i.e., sum of the amount of two consecutive rain events with their corresponding Q factor percentages.

Table A.1
Comparison of the response variability caused by the reduction treatments and the natural interseasonal response variability of the ambient treatment.

Season	Ambient treatment	Treatment with maximum effect	% change treatment	% change with previous season	% change Max./min. season
		A(µmol CO_2 m^{-2} s^{-1})			
SPR 09	9.56	Q50-F100 / 6.56	21.67%	-	-
SUM 09	0.24	Q100-F50 / 0.15	56.67%	**97.67%**	-
AUT 09	6.48	Q75-F50 / 2.98	53.00%	93.04%	49.99% / 51.18%
WIN 10	9.56	Q75-F75 / 7.41	20.33%	30.89%	30.26% / **70.66%**
SPR 10	13.61	Q50-F100 / 12.63	22.33%	30.26%	0.00% / **79.60%**
SUM 10	2.74	Q50-F50 / 1.87	28.67%	**79.60%**	**79.60%** / 0.00%
AUT 10	0.64	Q100-F50 / 0.22	39.82%	78.86%	**95.01%** / 0.00%
*WIN 11	11.2	Q75-F75 / 4.60	49.70%	**95.01%**	0.00% / **95.01%**
SPR11	10.77	Q75-F50 / 5.51	40.84%	40.18%	40.18% / **93.63%**
SUM 11	4.58	Q50-F50 / 2.15	53.28%	46.23%	54.90% / **87.01%**
*AUT 11	11.09	Q100-F75 / 5.26	50.63%	58.09%	-
		g_s (mol H_2O m^{-2} s^{-1})			
SPR 09	0.10	Q50-F100 / 0.07	28.01%	-	-
*SUM 09	0.012	Q100-F50 / 0.011	65.39%	85.78%	-
AUT 09	0.07	Q75-F50 / 0.03	43.14%	76.23%	59.08% / 38.92%
WIN 10	0.12	Q75-F75 / 0.09	26.52%	36.31%	31.12% / **69.78%**
SPR 10	0.18	Q75-F100 / 0.15	14.78%	31.12%	0.00% / **79.92%**
SUM 10	0.04	Q100-F75 / 0.03	14.07%	**79.92%**	**79.92%** / 0.00%
*AUT 10	0.02	Q75-F100 / 0.01	33.33%	45.26%	**85.85%** / 0.00%
*WIN 11	0.14	Q75-F75 / 0.05	58.44%	85.85%	0.00% / 85.85%
*SPR11	0.13	Q75-F50 / 0.05	59.58%	34.15%	34.15% / **84.17%**
*SUM 11	0.05	Q50-F75 / 0.02	54.65%	59.65%	60.45% / 61.24%
*AUT 11	0.12	Q75-F75 / 0.06	48.89%	52.92%	-
		IWUE (mmol CO_2/mol H_2O)			
SPR 09	0.08	Q100-F50 / 0.10	11.46%	-	-
*SUM 09	0.03	Q75-F75 / 0.04	81.33%	71.69%	-
*AUT 09	0.09	Q75-F75 / 0.13	28.30%	**73.01%**	0.00% / 20.04%
WIN 10	0.080	Q75-F75 / 0.083	12.59%	13.26%	13.26% / 13.51%
SPR 10	0.08	Q75-F75 / 0.09	16.07%	8.63%	15.56% / 9.45%
SUM 10	0.07	Q50-F75 / 0.10	23.61%	9.45%	20.04% / 0.00%

AUT 10	0.03	Q75-F100 / 0.08	50.96%	60.49%	68.51% / 0.00%
WIN 11	0.08	Q50-F50 / 0.11	27.05%	**62.31%**	15.54% / **62.31%**
*SPR11	0.08	Q50-F75 / 0.13	39.50%	23.98%	21.11% / 58.81%
*SUM 11	0.09	Q75-F100 / 0.15	29.92%	21.11%	0.00% / 68.51%
*AUT 11	0.10	Q50-F75 / 0.12	15.57%	14.68%	-
			F_v/F_m		
*SPR 09	0.61	Q50-F75 / 0.73	15.81%	-	-
SUM 09	0.50	Q75-F75 / 0.54	18.59%	17.54%	-
AUT 09	0.73	Q75-F100 / 0.74	3.57%	**30.63%**	3.41% / **21.73%**
WIN 10	0.72	Q50-F50 / 0.74	3.91%	1.49%	2.47% / **20.96%**
SPR 10	0.74	Q50-F75 / 0.75	3.30%	2.47%	0.00% / **22.79%**
*SUM 10	0.57	Q50-F75 / 0.64	10.94%	22.79%	22.79% / 0.00%
AUT 10	0.55	Q100-F75 / 0.59	13.29%	10.08%	22.35% / 0.00%
WIN 11	0.71	Q100-F50 / 0.74	4.37%	22.35%	0.00% / 22.35%
SPR11	0.68	Q75-F100 / 0.71	13.93%	6.11%	6.11% / 18.87%
SUM 11	0.67	Q75-F100 / 0.69	3.81%	10.59%	6.46% / 20.75%
*AUT 11	0.78	Q50-F75 / 0.79	2.93%	**13.62%**	-

* denotes seasons with significant responses to the treatments in the LMM models. Treatments with maximum effect are those that caused minimum seasonal *A* and g_s, and maximum seasonal IWUE and F_v/F_m. The fourth column gives the rate of change from the ambient treatment to treatment with the maximum seasonal effect. The fifth column gives the rate of change between the ambient treatment and its value in the antecedent season. The sixth column gives the rate of change from the ambient treatment to its seasonal maximum and minimum within hydrological years. The seasonal rates of change of the ambient treatment are highlighted in black bold when are significantly higher (p values < 0.05) than the intraseasonal rates of change caused by the rainfall reduction treatments (n = 3). Note that the % of change were not calculated with the values of columns two and three, but as the averages of the % of change per plot-size.

Table A.2
Comparison of the response variability caused by the reduction treatments and the natural interannual response variability of the ambient treatment.

Years	Ambient treatment	Treatment with maximum effect	% change treatments	% change between years
		A(µmol CO_2 m^{-2} s^{-1})		
09-10	7.51	Q75-F75 / 6.50	12.83%	19.89%
10-11	6.21	Q75-F75 / 3.62	40.30%	19.89%
		g_s (mol H_2O m^{-2} s^{-1})		
09-10	0.10	Q75-F75 / 0.07	21.12%	20.51%
10-11	0.08	Q50-F75 / 0.04	45.15%	20.51%
		IWUE (mmol CO_2/mol H_2O)		
09-10	0.073	Q75-F75 / 0.09	**17.40%**	**4.02%**
*10-11	0.071	Q50-F75 / 0.11	**31.91%**	**4.02%**
*Both years	0.072	Q100-F50 / 0.070	3.93%	4.02%
		F_v/F_m		
09-10	0.672	Q50-F75 / 0.70	3.41%	2.50%
10-11	0.667	Q75-F100 /0.668	2.49%	2.50%

* denotes hydrological years with significant responses to the treatments in the LMM models. Treatments with maximum effect are those that caused minimum annual *A* and g_s, and maximum annual IWUE and F_v/F_m. "Both years" in IWUE refers to the treatment with the minimum biannual average. The fourth column gives the rate of change from the ambient treatment to the treatment with the maximum annual effect. The fifth column gives the rate of change from the ambient treatment to its value in the other year. The inter-annual rates of change of the ambient treatment are highlighted in red bold when are significantly higher (p values < 0.05) than the intra-annual rates of change caused by the rainfall reduction treatments (n = 3). Note that the % of change were not calculated with the values of columns two and three, but as the averages of the % of change per plot-size.

.....y seguiré caminando a media ladera.....